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# The Journal of PARASITOLOGY

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# A STUDY OF THE *IN VITRO* ACTIONS OF ANTISERUMS ON THE CERCARIAE OF CERTAIN BIRD SCHISTOSOMES*

J. R. HENDRICKS AND W. W. CORT

It has been known for many years that various serologic phenomena, such as agglutination and precipitation can be demonstrated by placing certain infectious agents in homologous antiserum. However, it was only recently that the larval stages of schistosomes were used in such studies.

Papirmeister and Bang (1948) described the development of an "enveloping precipitate" around the cercariae of *Schistosoma mansoni* when exposed to fresh antiserums of man and monkey. This precipitate was formed within 3 hours, and shortly afterward the cercariae died and began to disintegrate. Since the reaction did not occur in serum from uninfected man and monkey, it was thought to be a specific antigen-antibody reaction. As a sequel to this study, Liu and Bang (1950), using inactivated (56° C for 30 minutes) serum from monkeys infected with *S. mansoni,* demonstrated an agglutination of the cercariae. Their explanation for the difference between this reaction and the previously reported precipitate-formation was the inactivation of the serum. They suggested that different antibodies were involved in the two reactions.

A third, strikingly different, reaction of schistosome cercariae in antiserum was first described by Vogel and Minning (1949a). This phenomenon, the formation of a membranous pericercarial envelope, was called the "Cercarienhüllenreaktion" (CHR). They noted that cercariae of *S. mansoni,* when placed in fresh or inactivated specific antiserum, formed immediately a firm, compact sheath. The reaction was shown to occur also in dermatitis-producing cercariae of *Trichobilharzia* sp. when they were suspended in serums from dogs and rabbits infected with *Schistosoma japonicum,* from a patient with *S. hematobium,* and from patients with *S. mansoni* (Vogel and Minning, 1949b).

Stimulated by the differences in the previous results Stirewalt and Evans (1955) conducted a study to compare all of these reactions and to determine their relationship to the immune response in the infected hosts. They tested cercariae of *S. mansoni* in serums from various infected and uninfected mammals. The precipitation reaction was shown to be unrelated to schistosome infection, since it could be demonstrated in fresh serums of various uninfected animals. Moreover, they concluded that the agglutination and formation of the pericercarial envelope are manifestations of the same phenomenon, the reaction obtained being dependent upon the

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dilution of the antiserum. It was suggested further that neither of these reactions is specific, since cross-reactions have been observed with the cercariae of *S. japonicum* in *S. mansoni* antiserum.

A better understanding of the CHR phenomenon was made possible by the observation by Gustafson (Stirewalt and Evans, 1955) that the cuticle of the cercariae is not involved directly in the envelope formation, since the cuticular spines were seen to remain and the spine impressions were present on the inner surface of the envelope. It appears from the observations of Kruidenier and Stirewalt (1955) that the pericercarial envelope has its origin in a fine extra-cuticular film around the cercariae. From their observations of cercariae of *S. mansoni* in specific antiserum, it is clear that the envelope is produced from this film by combination with antiserum elements. Analysis of the film material showed that it is formed from secretions of the "penetration-gland" complex. According to these workers, these secretions are produced by the immature cercariae at the time of their emergence from the sporocyst; the discharges of the cercariae after emergence from the snail tissues do not contribute to this extra-cuticular film nor to the formation of the envelope.

It is clear from the above discussion that much has been learned recently concerning the *in vitro* action of antiserums on cercariae of certain schistosomes. The present study was designed with the hope of obtaining results that would help to explain better the mechanisms of these various reactions.

## MATERIALS AND METHODS

The cercariae used for infecting and for testing were obtained from infected snails collected in the Douglas Lake region of northern Michigan: The cercariae of *Trichobilharzia elvae* (Miller, 1923) from *Lymnea stagnalis,* those of *T. stagnicolae* (Talbot, 1936) from *Stagnicola emarginata,* and those of *T. physellae* (Talbot, 1936) from *Physa parkeri.* They were used within 12 hours after being shed.

Rabbits and ducklings were used in the experimental infections to produce antiserum. Rabbits, 2 months old at the beginning of the experiment were exposed, 1 to each species of cercaria, either by placing water containing large numbers of cercariae on the freshly shaven abdomen, or by immersing the ears in the cercarial suspensions. The exposure time varied from 10 to 15 minutes and in every case sites of penetration could be observed. The rabbits were given 8 exposures at intervals of 1 to 4 days. Each animal was exposed to only 1 species of cercaria.

Domestic ducklings, a few days old at the beginning of the experiment, were exposed 8 times at 2- to 4-day intervals to large numbers of cercariae, each animal to only 1 species of cercaria. The exposure time varied from 20 to 30 minutes. Of the 5 ducklings exposed to the cercariae of *T. elvae,* 3 passed eggs in the feces, 1 on the 19th and 2 on the 20th day after initial exposures. Of the 3 ducklings exposed to the cercariae of *T. physellae,* eggs were found in the feces of only one; this was on the 38th day. Of the 6 exposed to the cercariae of *T. stagnicolae* no eggs were found in the feces within the observation period. Nevertheless, it is certain that there was penetration of large numbers of cercariae.

The antiserums used in the comparative study were obtained from 1 rabbit exposed to *T. elvae,* 1 to *T. physellae,* and 1 to *T. stagnicolae;* the duckling which passed eggs of *T. elvae* after 19 days, the one which passed eggs of *T. physellae* after 38 days, and one with comparable exposures to *T. stagnicolae.*

The experimental animals were bled 8 weeks after initial exposure. The blood, obtained by heart puncture, was placed in a 10-cc tube, allowed to clot at room temperature, ringed, and placed in the refrigerator overnight. After centrifugation the serum was pipetted into smaller tubes, inactivated at 56° C for 30 minutes, and stored at 6–8° C until used in the tests. Serum from uninfected hosts was obtained and stored in this same manner.

Tests were performed by mixing 0.5 cc of the serum, undiluted and in 2-fold dilutions of filtered lake water, with 0.5 cc of the water containing 50–75 cercariae. By this method, the final dilutions, expressed in ratio of parts of serum to total volume, in which the cercariae were contained, were 1:2, 1:3, 1:6, 1:12, . . . 1:768. The initial mixtures were made in small watch glasses and covers were sealed in place with vaseline. For microscopic examinations, slide preparations with supported, sealed cover slips were made at the desired time intervals. Critical readings were made after 5 minutes, 2 hours and 24 hours. The series for each species consisted of cercariae in 2-fold dilutions of serum from animals infected with the homologous species, the same dilutions of serum from animals infected with each of the other 2 species, and the same dilutions of serum from uninfected animals. In addition, controls, using filtered lake water in place of the serum dilutions, were included for each species.

## RESULTS

Using the appropriate controls, mentioned above, a total of 40 tests was made with each of the 3 species of cercariae in homologous antiserum from both rabbits and ducklings and in the 2 heterologous antiserums from both host animals. The reactions of the 3 species were similar in various dilutions of the respective homologous antiserum, but in all cases the reactions were noted sooner and in higher titers in rabbit antiserum than in duckling antiserum. The reactions of the 3 species of cercariae were indistinguishable whether in homologous antiserum or in either of the 2 heterologous antiserums. These cross-reactions indicate their non-specificity and support the suggestion of other workers, that a group-specific antigen(s) is involved. In view of the similarity of these reactions, the descriptions will be limited to the changes observed in the cercariae of *T. elvae* in representative dilutions of homologous antiserum (1:2, 1:12, and 1:48).

*Tests with the cercariae of* T. elvae *in rabbit serums.*—Cercariae placed in various dilutions of inactivated normal rabbit serum remained actively motile for the 24-hour observation period. Likewise, the cercariae in the preparations made with lake water were also unchanged in microscopic appearance within this time.

In the 1:2 dilution of antiserum, the reaction of the cercariae began almost immediately. Within 2 minutes, the exterior margin of the tail began to have a wrinkled, sheath-like appearance, with the furci curling anteriorly. This did not appear to alter the activity of the cercariae. After 15 minutes a thin line appeared, parallel and external to the cuticle, at the region of the ventral sucker. By this time the tail was completely ensheathed. After the first hour, the swelling of the sheath around the body had increased and its exterior margin had become separated further from the cuticle. The space between the sheath margin and the cuticle increased anteriorly and posteriorly at about the same rate. At this stage, the external margin of the body sheath became continuous with the sheath formed earlier on the tail. After 2 hours, the maximum reaction in this dilution was reached (Fig. 1), and no

further change was noted during the 24-hour period. The sheath became wrinkled in appearance and the swollen area was about half as wide as the cercarial body. Throughout the observation period, the cercariae remained actively motile within the sheaths. In some, the tails separated from the bodies, after which both remained motile within the sheath. A number were seen to break through the anterior end of their sheaths and become free in the surrounding medium. These escaped cercariae, always without the tails, soon showed a mucoid, stringy precipitate at the anterior tip (Fig. 2).

A gradual change was observed in the general appearance of the cercariae with greater serum dilutions. In the 1:12 dilution, this change was very striking (Fig. 3). The early reactions of the tail were essentially as those described above, with less curling of the furci. The beginning of the body sheath was delayed for about 20 minutes and progressed posteriorly more slowly than in the more concentrated serum. Also, the sheath did not become as thick and, within 24 hours, had not covered the anterior end. In addition to these partial sheaths, mucoid material appeared at the anterior ends of the cercariae. Apparently, this material was responsible for the adhesion of the cercariae to one another, and to the slide and cover slip, which frequently occurred in the preparation. Although the activity of the cercariae was not reduced within 24 hours, progress was impeded by this sticky mass. This mucus material apparently had become dispersed in the fluid, causing the sheaths to adhere to one another while the cercariae remained active with their individual partial sheaths. This adhesion was not firm, since the cercariae in the original mixture could be separated easily.

In even more dilute preparations (1:48, specifically), the tail cuticle wrinkled but no sheath was observed. The cuticle on the body of the cercariae remained unchanged, but within 20 minutes large masses of mucoid material were observed orally. In the slide preparations, this material caused the cercariae to adhere to the slide and cover slip, and to each other when contact was made. The 1-hour slide preparation showed that the mucus had become dispersed in the medium, and that the cercariae had become covered with this material and with adhering bits of debris (Fig. 4). Large amounts of mucus were still present at the anterior ends. Examination of the original watch glass preparation after 12 hours showed a compact mass of cercariae, which were still living as evidenced by motility. This condition, comparable to the agglutination described by others, persisted even after the cercariae were pipetted onto the microscopic slide. At this dilution there was no reaction resembling the pericercarial envelope formation. The degree of reaction diminished with the increasing dilutions of antiserum. In the 1:384 dilution the cercariae were not distinguishable from those in the controls.

*Tests with the cercariae of* T. elvae *in duckling serums.*—In preparations of cercariae with various dilutions of inactivated normal duckling serum and in those with the lake water, no change was observed throughout the 24-hour period. The cercariae remained actively motile without noticeable surface alteration.

Cercariae in the 1:2 dilution of antiserum behaved in a manner indistinguishable from those in the 1:12 dilution of rabbit antiserum and the appearance of the external surface was similar (Fig. 3). Likewise, the reaction in the 1:12 dilution of duckling antiserum was similar to that in the 1:48 dilution of rabbit antiserum. However, in the 1:48 dilution of duckling antiserum, the cercariae were actively motile, free swimming, and even after 24 hours were indistinguishable from the

controls. Thus, it appears that the serum elements responsible for the observed reactions were present in greater concentration in the rabbit antiserum. This is not surprising, since it is known that rabbits respond to antigenic stimulation more effectively that do most other animals.

## DISCUSSION

The results of the present study show that serum from rabbits and ducklings, exposed repeatedly to the cercariae of *T. elvae, T. physellae* and *T. stagnicolae,* cause *in vitro* an agglutination of the cercariae and the formation of a pericercarial envelope (CHR). In general, the CHR phenomenon observed here was similar to that described previously for the human schistosomes (Vogel and Minning, 1949a, 1949b; Stirewalt and Evans, 1955) and for a species of *Trichobilharzia* (Vogel and Minning, 1949b). The time of appearance of the firm, compact sheath in low dilution serum (20 minutes in the 1:2 dilution) was about the same as in the experiments of Vogel and Minning (1949a). However, Stirewalt and Evans reported that a longer time (1 hour) was necessary for the formation of the firm sheath. Furthermore, they found oral secretions of large amounts of sticky material, preceding the sheath formation. In the present work, the cercariae with the complete sheath did not form an oral precipitate prior to the sheath formation. Neither of the previous reports showed cercarial tail involvement comparable to that in the present study. Vogel and Minning noted a delicate membrane on the upper surface of the tail in a very few cases, while Stirewalt and Evans noted, in concentrated antiserum, complete tail sheaths which spread from the previously formed body sheaths. In the present study, the tail was first to show a reaction in the antiserum, which was noted even in the 1:192 dilution. This was followed, in the low dilutions, by the beginning of the body sheath at about the level of the ventral sucker and, eventually, by the joining of the 2 sheaths.

Both of the earlier papers, mentioned above, indicated varying degrees of sheath formation; that is, the quickly formed, hardened sheath, and the thinner sheath, formed later and having a sticky surface. In the present work, the sheath, whether complete (covering entire body) or partial (covering posterior part of body), seemed always to have a hardened exterior surface. Instances in which the margins of the partial sheaths were not entirely free of adhering particles, were believed to be the result of the sticky, oral secretions being dispersed into the surrounding fluid. The preparations in which the complete sheaths formed immediately and some of the cercariae escaped from the anterior end (Fig. 2), showed some of this apparent stickiness since the cercariae sometimes became attached loosely to each other. Here again, this was believed to be the result of the oral secretions after the cercaria had escaped from the sheath, since a similar precipitate was observed at the end of cercariae in the high dilutions of the antiserum.

Agglutination of cercariae, as observed in the present study, appears to be the result of oral secretions of the cercariae when placed in weakly reacting (dilute) antiserum. This can be noted in the sequence of reactions in Figures 1–4. In the low dilutions, in which the envelope formed immediately, the oral mucoid secretion was not observed in the surrounding medium nor on the exterior of the sheath. However, some of the ensheathed cercariae contained such material within the anterior end of the sheath. In the next higher dilution, in which the sheath was

formed on the tail and posterior half of the body, but not over the anterior end, large quantities of this secretion were observed. This material was seen to be sticky, causing the cercariae to adhere to the slide, coverslip, and to each other. The most striking change in this appearance of the cercariae was in the high dilutions, in which there was no clearly formed envelope. Cercariae in dilutions of 1:48 produced the most profuse oral secretion, some of which soon became dispersed in the surrounding medium. The cuticle of these cercariae became coated with the sticky material as well as with bits of debris from the surrounding fluid (Fig. 4). The cercariae remained motile but progress was impeded and they adhered to one another. In still higher dilutions of antiserum, this reaction diminished gradually, so that in the 1:384 dilution of rabbit antiserum, the cercariae were indistinguishable from those in the normal serum controls.

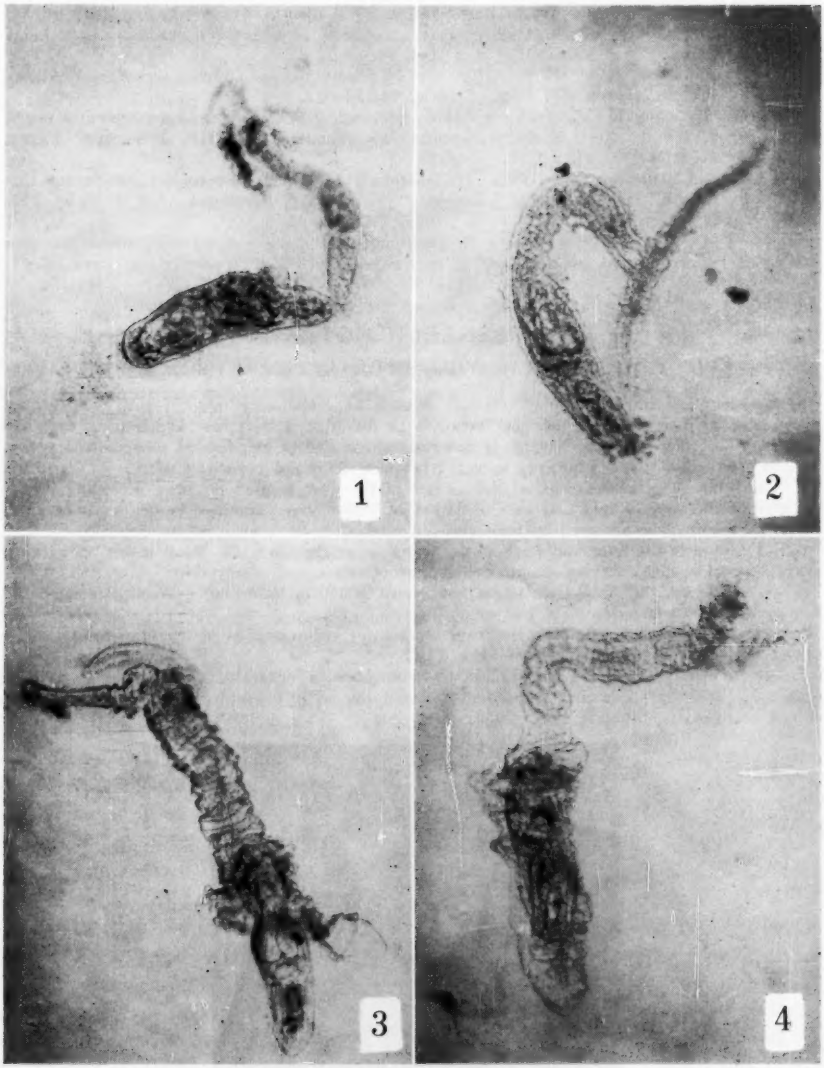
The evidence presented confirms earlier reports that the factors in antiserum responsible for agglutination of cercariae and for the pericercarial envelop formation are present in the same serum. However, it appears that different properties of the cercariae are responsible for each reaction. The CHR is clearly a result of the fine extra-cuticular material present on the cercariae as explained by Kruidenier and Stirewalt (1955), while the agent responsible for the agglutination seems to arise from the oral secretions of the cercariae. The concentration of the serum elements apparently determines which of the reactions will be evident. In the presence of a low dilution of a strongly reacting antiserum, the "Cercarienhullen-reaktion" takes place quickly and prevents the oral secretion from coming in contact with the serum elements. On the other hand, if the membrane is not formed over the anterior end immediately, or at all as in the case of greatly diluted antiserum, the oral secretions are responsible for the final agglutination reaction.

## SUMMARY

The *in vitro* activity of cercariae of *Trichobilharzia elvae, T. physellae,* and *T. stagnicolae* was observed in serums of rabbits and ducklings with homologous and heterologous infections, and compared with the effects of serums from the uninfected host species. Inactivated (56° C for 30 minutes) serums from the uninfected hosts produced no detrimental effect on the cercariae in 24 hours at room temperature. Low dilutions of rabbit antiserum caused a characteristic pericercarial envelope formation (CHR). In more dilute rabbit antiserum and low dilutions of duckling antiserum only a partial envelope formed and a mucoid secretion appeared at the anterior end. In higher dilutions of the antiserums of both hosts, the cercariae did not form recognizable envelopes but did secrete large amounts of material from the oral openings.

It is suggested that the phenomena of envelope-formation and agglutination arise from different reacting principles. In strong antiserum, the envelope forms immediately and, as has been pointed out by others, seems to have its origin in the fine extra-cuticular material present on the cercariae. In weaker antiserums, the envelope does not form over the anterior end of the cercariae. In these the oral secretion, which appears to be sticky, seems to be responsible for the tendency of the cercariae to adhere to the slide and to one another. In the weakest of the reacting antiserums, no envelope is seen to form but profuse oral secretion occurs and becomes dispersed in the surrounding medium. This is apparently responsible for the firm agglutination of the cercariae.

PLATE I




EXPLANATION OF FIGURES

Figs. 1–4. Cercariae of *Trichobilharzia elvae* suspended in specific antiserum.
FIG. 1. After 2 hours in the 1:2 dilution.
FIG. 2. After 6 hours in the 1:2 dilution.
FIG. 3. After 2 hours in the 1:12 dilution.
FIG. 4. After 2 hours in the 1:48 dilution.

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## RESEARCH NOTE

### METHOD FOR ISOLATING SCHISTOSOME GRANULOMAS FROM MOUSE LIVER

An early and intense cellular reaction in mice experimentally infected with *Schistosoma mansoni* takes place around the eggs retained in the liver and typical granulomas are then formed. The study of such elements is generally performed in histological sections and preparations from small liver fragments squeezed between slide and cover-slip.

The counting of granulomas on the surface of the liver is used for drug screening in experimental therapy. Rapid and easy isolation of schistosome granulomas of the liver from infected mice may be achieved by the following method:

1—Remove the liver and wash in tap water. Cut the liver into small pieces, care being taken to remove the adhering diaphragm, ligaments, veins and biliary ducts.

2—Transfer the small liver pieces, suspended in 150 ml saline, to a Waring blender and homogenize for 3 minutes.

3—Pour the material into cylindrical glasses for sedimentation of the granulomas which are easily observed as small granules (Fig. 1).

4—Wash the granulomas by repeated sedimentation. The isolated granulomas may be stored in 10 percent formalin for subsequent counting. The number of granulomas is determined by direct counting in Petri dishes.

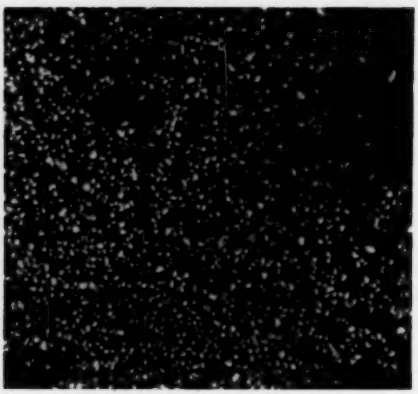

FIGURE 1—Schistosome granulomas isolated from mouse liver, 8 weeks after infection. Natural size.

This method is obviously applied only for the isolation of full developed granulomas which appear in the mouse liver 8 weeks after infection.—J. PELLEGRINO AND Z. BRENER, *Instituto Nacional de Endemias Rurais, Centro de Pesquisas de Belo Horizonte, Brazil.*

# PENETRATION OF HOST SKIN BY CERCARIAE OF *SCHISTOSOMA MANSONI*. I. OBSERVED ENTRY INTO SKIN OF MOUSE, HAMSTER, RAT, MONKEY AND MAN

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A more thorough understanding of the means of entry, and the pattern and mechanism of migration of schistosomes through the skin of definitive hosts is essential to a comprehensive knowledge of host-schistosome relations. Although entry has been described in the skin of the various animals listed in Table I, the need for further study of this first step in the infection is accentuated by the disagreement over the point of entry of cercariae which has existed among workers on the schistosomes and which remains largely unresolved. Therefore, a comparative study was undertaken of the frequency of cercarial entry into unshaved skin of a variety of hosts, supplemented by observation of the means of entry and the pattern of peripheral migration. This paper reports the results of direct observation of entry

## MATERIALS AND METHODS

Entrance of cercariae of *S. mansoni* was observed in the various types of skin of the following animals: ear, body and tail skin of adult hairless mice,* tail skin of adult haired (C3H) mice, body skin of 1- to 2-day old and 4- to 5-day old mice and of 5-day old rats, body skin of adult golden hamsters, body skin of adult macaque monkeys, and skin from the abdomen, the finger tip, and the dorsum of the basal joints of the third finger and the great toe of adult human beings.

Cercariae were those of Puerto Rican *S. mansoni* maintained in mice and hamsters and in laboratory-reared *Australorbis glabratus* of Puerto Rican origin. They were collected from 6 or more snails between 10:00 a.m. and 1:00 p.m. on the day of use and applied to skin within 5 hours of the beginning of emergence.

Entry of cercariae was observed with a dissecting microscope with 10× oculars and 1×, 3×, and 6× objectives and reflected light, and a compound microscope with 12× oculars, a 16-mm objective, and transmitted light. Reflected light was from 3 Spencer microscope lamps arranged in a semicircle and equipped with cooling cells.

For routine observation, skin was observed *in situ* or excised from anesthetized hosts, cleaned of underlying tissue and stretched across a circular opening in a piece of thin sheet cork. Cercariae were applied to skin, singly or in groups, under the dissecting microscope, but, wherever possible, further observation was with higher magnification.

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The opinions or assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

* Received from Dr. Margaret Deringer, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

Table I. - Summary of Reported Cercarial Entry Sites in Skin of a Variety of Hosts

| Author | Host | Skin | Parasite |
|---|---|---|---|
| | **Through Stratum Corneum** | | |
| Leiper 1915 | Young mouse | Body | *S. mansoni* |
| Lutz 1919 | Rat, young pig, guinea pig | Body | *S. mansoni* |
| Vogel 1932 | Man | Forearm | *Cercaria elvae* |
| Standen 1953 | White mouse | Shaved abdominal | *S. mansoni* |
| | **By Hair Follicles** | | |
| Iturbi and Gonzalez 1919 | Rat and guinea pig | Body | *S. mansoni* |
| Cort 1921 | Man | Forearm | *C. elvae* |
| Christenson and Greene 1928 | Man | Forearm | *C. elvae* |
| Fairley *et al.* 1930 | Guinea pig | Depilated abdominal | *Schistosoma spindale* |
| Watarai 1936 | Domestic fowl | Groin | *S. japonicum* |
| Stirewalt 1953 | C3H mouse | Tail | *S. mansoni* |
| | **Both by Hair Follicles and Through Stratum Corneum** | | |
| Miyagawa 1912 | Dog | Body | *S. japonicum* |
| Narabayashi 1913 | Mouse and rat | Body | *S. japonicum* |
| 1915 | Mouse, dog, man | Body | *S. japonicum* |
| 1916 | Man and experimental animals | Body | *S. japonicum* |
| Vogel 1930 | Man | Forearm | *Cercaria ocellata* |
| Koppisch 1937 | Rabbit | Shaved abdominal | *S. mansoni* |
| Pinto and Almeida 1948 | Dog | Abdominal | *S. mansoni* |
| Gordon and Griffiths 1951 | Mouse | Abdominal | *S. mansoni* |

To facilitate observation, cercariae were often stained supravitally for 3 minutes with a saturated aqueous solution of purpurin indicator (C.I. #1037, Conn, 1953) diluted with an equal volume of cercarial suspension. Purpurin stained the preacetabular glands selectively and thus marked the cercariae for accurate observation, but did not interfere with their entry into skin.

Records were kept of the number of cercariae penetrating each skin (Table II), and, for as many penetrants as possible, of the sites used (Table III) and of the length of time spent in exploration and entry (Table IV). The exploratory period was from the time the parasite was pipetted onto the skin until it oriented itself vertically or obliquely to the skin, attached orally to the surface, and began to elongate and contract in a successful entry attempt. The entry period was that from the beginning of the successful entry attempt to the disappearance of the aboral end of the body of the cercaria under the *stratum corneum*. Cercariae not entering within 25 minutes were usually immobile and were called failures.

Not only was entry into skin clearly observed, but in thin skin the schistosomulae were followed for as long as they remained in the upper tissue levels.

## OBSERVATIONS

Overt cercarial reactions were similar on skin of all the hosts except the young rat, as recorded later. They were the same in all observed respects when the host was restrained; anesthetized with ether, chloroform, or nembutal; dead; or even when the skin had been excised from anesthetized or dead hosts. Cercariae continued to enter excised skin for as long as 24 hours after the host's death.

*Ear skin of adult hairless mice* was especially favorable for observation of penetration of skin. The skin surface was not deeply wrinkled, but many long shallow furrows intersected at frequent intervals. Tiny papillae produced a knobby surface interrupted by the larger eminences which surrounded the numerous follicular canal orifices. Of these some were patent; others surrounded the stumps of hair shafts or were clogged with sebum. The observations recorded during entry of cercariae into this skin may serve as a basis for comparison with other skins.

Cercariae applied to skin in a small drop of water usually swam briefly and then settled onto the skin surface over which they looped for variable periods of time

Table II - Skin of Hosts Ranked in Descending Order of Percent Entry by Cercariae of *Schistosoma mansoni*

| Skin | Number of Hosts | Number of Skins | Total number of Cercariae | Percent Cercariae Entering |
|---|---|---|---|---|
| Adult haired (C3H) mouse tail | 9 | 9 | 77 | 91 |
| Adult monkey body | 3 | 3 | 17 | 88 |
| Adult human body | 2 | 3 | 21 | 86 |
| Adult hairless mouse ear | 4 | 8 | 37 | 78 |
| Adult hamster body | 4 | 11 | 113 | 78 |
| Adult hairless mouse body | 9 | 10 | 55 | 73 |
| 4-5 day mouse body | 3 | 4 | 51 | 73 |
| 1-2 day mouse body | 9 | 11 | 88 | 65 |
| Adult hairless mouse tail | 4 | 5 | 31 | 62 |
| 5 day rat body | 3 | 5 | 32 | 34 |

Table III. - Percentage of Cercariae of *Schistosoma mansoni* Entering Skin of the Hosts by the Sites Listed

| Skin | Cercariae Observed | Wrinkle Wall | Annular Tail Groove | Skin Scale | Sebum Mass | Distal Hair-skin Angle | Follicular Orifice | Used Entry Site |
|---|---|---|---|---|---|---|---|---|
| Adult hairless mouse ear | 27 | 26 | | 7 | | 4 | 59 | 4 |
| Adult hairless mouse body | 36 | 56 | | 19 | | | 25 | |
| Adult hairless mouse tail | 24 | | 4 | | | | 96 | |
| Adult haired mouse tail | 63 | | 13 | | | 5 | 82 | |
| 1-2 day mouse body | 56 | 86 | | 2 | | | | 12 |
| 4-5 day mouse body | 37 | 81 | | 3 | | 8 | 5 | 3 |
| Adult hamster body | 88 | 16 | | 15 | 9 | 18 | 42 | |
| 5 day rat body | 11 | 55 | | | | | 27 | 18 |
| Adult monkey body | 15 | 46 | | | | 7 | 27 | 20 |
| Adult human body | 26 | 100 | | | | | | |

(Table IV) by attaching alternately with the oral and ventral suckers. When unattached, the oral end of the body constantly probed into every irregularity encountered.

Wherever the oral sucker was attached and the ducts of the purpurin-stained preacetabular glands were everted through its rim, tiny pink deposits were left. Thus it was possible to follow the progress of a cercaria by its "tracks", which formed as the droplets of secretion extruded onto the skin, absorbed water, swelled, and became sticky.

Table IV. Exploratory and Entry Times for Cercariae on the Skin of Different Hosts of *Schistosoma mansoni* Ranked in Order of Increased Entry Time

| Skin | Number Cercariae Timed | Exploratory Time in Minutes | | Entry Time in Minutes | |
|---|---|---|---|---|---|
| | | Av. | Range | Av. | Range |
| 1-2 day mouse body | 56 | 1.8 | 0.3-5 | 2.7 | 0.7-11 |
| Adult hairless mouse body | 30 | 2.5 | 0.6-9 | 3.0 | 0.8-11 |
| Adult hairless mouse ear | 13 | 2.8 | 0-10 | 3.1 | 0.8-8 |
| Adult haired (C3H) mouse tail | 32 | 0.7 | 0-3 | 5.5 | 1.5-15 |
| 4-5 day mouse body | 30 | 2.0 | 0-13 | 5.8 | 1.2-17 |
| Adult monkey body | 11 | 2.2 | 0.5-3 | 5.8 | 1.8-12 |
| 5 day rat body | 11 | 4.4 | 1.5-14 | 6.9 | 2.0-17 |
| Adult hairless mouse tail | 19 | 0.8 | 0.2-6 | 6.9 | 2.7-12 |
| Adult human body | 21 | 0.4 | 0-1 | 7.2 | 3.1-17 |
| Adult hamster body | 47 | 2.3 | 0.5-10 | 7.3 | 2.0-19 |

Some cercariae entered at the first site selected; others tried several sites before entrance was achieved. Walls of wrinkles including the bases of follicular eminences, points of skin scale attachment, distal hair-skin angles, orifices of follicular canals, and used entry sites served as passages into this skin (Table III). Five of the 27 penetrants attempted entry at the edge of the drop of water.

Having picked a site, a penetrating cercaria released the acetabulum from the skin surface, attached closely with the oral sucker and assumed a vertical or oblique position with relation to the surface. At this time the cercarial body was elongated and slender and the muscular oral sucker was thrust into the entry site. Ramming motion and partial eversion of the sucker brought the openings of the ducts from the acetabular glands into contact with the skin. Through these, the gland contents were secreted onto the host tissues. Alternate contraction and elongation of the body and energetic movement of the tail accompanied the thrusts of the oral sucker. These activities were not synchronized and considerable elongation of the gland ducts was sometimes required. Under these conditions, the great dorsal arches in the ducts in the vicinity of the retracted oral sucker disappeared when the sucker was everted and the body elongated.

Each of these activities played an important part in penetration. Elongation of the body at the time the oral sucker probed initially into the entry site permitted the cercaria to push into very small crevices. Then, closely attached and buried orally, the body was contracted so the diameter increased, with the result that the breach in the host tissue was enlarged. This sequence of slenderizing the body while the oral tip was being thrust deeper, and then contracting the body and pouring out glandular secretion constituted the regular means of enlarging the entry pore for gradual penetration.

Constant tail activity gave added forward thrust. This motion of the tail differed from the use of the appendage in swimming when the larva was usually pulled through the water. When pushing into skin, the tail vibrated as a whip with the distal end freely lashing as it was contracted and elongated. So much propulsive force was produced in this way that during several prolonged entries the proximal end of the tail was observed to slip around the aboral end of the body and ensheath it. Sometimes the tail was lashed around the body so the furci neared or touched the oral end; at other times it was held curved around over the body as the abdomen of a scorpion, and quivered. Occasionally the furci were used to push against the water or the skin surface. Tail thrust was not a necessity, however, inasmuch as two tailless cercariae were able to penetrate, though only very slowly. Usually tailless cercariae failed to penetrate.

The initial breach and traverse of the *stratum corneum* was especially time-consuming. Once the oral end of the body had progressed beyond the corneal layers and turned to parallel the surface, the parasite slipped quickly into the skin. The *stratum corneum* fitted closely around the body of the cercaria as it entered. After entry, the pore appeared as a crater with an inner diameter of about 10μ and an outer of about 30μ. The elevated edges of the lesion were pink when the cercariae had been stained with purpurin. As a rule in this skin, the tail was detached as the aboral end of the body slid under the *stratum corneum*, so that it plugged the entry pore. Here it often continued to quiver for some time. Sometimes it was detached earlier and "swam" away as if the body were still attached. Very occasionally it was pulled under the surface, but only up to the furci.

During these observations cercariae were never seen to rotate on the long axis as they penetrated; rather the oral attachment appeared to be a firm one and to hold the cercaria tightly in position. This is contrary to Grandjean's observations of cercariae of *S. mansoni* rotating rapidly on the long axis as they penetrated frog skin (Gordon and Griffiths, 1951).

Twenty-two percent of the cercariae were unable to penetrate this skin. These appeared to exhaust their potentialities for entry quickly, and looped over the surface, made further ineffective attempts at entry, or swam or hung from the surface film. Finally, they lost the power of locomotion, and lay twitching convulsively for a time before death. Failure to penetrate sometimes resulted from an unfortunate choice of entry site, such as the point of attachment of a skin scale; e.g., several cercariae moved through the scale, instead of under the *stratum corneum*. Sometimes entry failures seemed to be the result of a lack of awareness of the proximity of skin, since some cercariae swam continuously or simply lay quiescent on the skin. Other cercariae attempted to enter but were unable to attach to the site orally; or, after attachment, made no progress into the skin. In these, the preacetabular glands and ducts were still brightly stained and apparently full, indicating that exhaustion of glandular material was not necessarily a contributing factor. Gradual loss of the purpurin-stained contents of these glands, however, accompanied skin entry, though paling of color could not be correlated consistently with the rate of progress into skin.

Thirty-seven single, consecutive cercariae were observed attempting entry on this skin. The average and range of times spent in exploration and in actual entrance are listed in Table IV. Multiple exposures differed in that when many cercariae

were applied together to the limited skin area covered by a small drop of water several often collected at one entry site and penetrated side by side. Whether or not this was purely chance was not ascertained. Such collaboration resulted in the faster entry of a greater proportion of cercariae.

Cercarial movement within ear skin could be followed for as long as 2 hours. Unless entry had been unusually slow, both the preacetabular glands and their ducts retained some purpurin stain and marked the migrant for observation. As the glands and ducts paled, the ventral pair appeared to lose color faster than the dorsal. The pink droplets secreted as the oral sucker was thrust forward identified the migration tunnel by their movement in the liquid medium as the parasite jostled them. The pink stain could sometimes be observed as it spread between the host cells in a semicircular area with radii as long as 50 microns from the oral sucker. Within the migration tunnel, the schistosomulum moved freely, but the range of motion was plainly limited by the walls of the tunnel, which reacted as a gel to probing by the parasite.

Exploratory movements of the mobile oral end of the body accompanied progressive motion which appeared as a smooth gliding activity resulting from a combination of alternate attachment of the suckers and elongation and contraction of the body. When progress was slow, oral exploration was intensified. At such times the schistosomulum often backed in the tunnel and attacked in a new direction.

The path was fortuitous, the parasite simply pushing into areas of low resistance encountered during exploration. The direction was serpentine, circular or spiral, or, more rarely, straight. Though generally it lay in a plane parallel to the skin surface during this early period of migration, occasionally schistosomula encountered hair follicles and followed them into the deeper tissues. A few moved up to the surface, but pulled the oral end back into the skin and continued to migrate.

The distances travelled in the skin within 30 minutes depended upon the difficulties encountered by individual migrants. If no resistant areas were encountered, the tunnel could be traced back to the point of entry as far as 1 mm away. Most migrants travelled more slowly. Some covered as little as 0.2 mm in 30 minutes.

Observation of entry into skin of an ear which had been removed from the mouse and stored at 8°C for 24 hours revealed no differences in cercarial behavior from skin removed immediately before the application of cercariae or still *in situ*. Locomotion in the stored skin, however, continued only for about 8 minutes, after which all visible motion of the schistosomula ceased.

*Body skin of adult hairless mice* has been described by Grüneberg (1952). It differs in many respects from the body skin of haired mice, but was substituted for it because satisfactory observation of cercariae on the latter was hindered by the mat of fine hair which could not be removed without injury to the *stratum corneum*.

Hairless mice of the age used had nearly denuded bodies with only the vibrissae and a few scattered disoriented hairs remaining. With magnification, the skin surface appeared as a rough mosaic of rectangular to circular areas of conical eminences, each topped by a follicular orifice (Fig. 1A). These were in rows and were separated by deep primary wrinkles (B) partially obliterated in Figure 1 by stretching of the skin. Shallow secondary wrinkles intersected freely over the entire surface. Some of the follicular orifices appeared to be patent and to function as sebaceous ducts, while others surrounded fine hairs, remnants of hair shafts, or

sebum plugs. Others appeared to be covered by a thin layer of *stratum corneum*. Many irregularities in the form of cystic enlargements, scuffed regions and lesions roughened the surface. Data on cercarial entry are given in the tables.

Sensory perception of the activities of one cercaria by another appeared to vary with individuals. Some passed close by cercariae in the process of penetrating without apparent awareness, while others seemed to sense such activity and deliberately to direct their movements toward them and to enter in the same area.

Cercarial tails were generally pulled into the migration tunnel in this skin. They were cast soon thereafter and remained in the tunnel near the entry pore.

Insofar as could be observed, the description of early migration in this skin was similar in all respects to that in ear skin. Within 15 minutes after entry those schistosomula which continued progressive motion moved about 1.8 mm. Some encountered barriers which slowed their progress, or ceased locomotion and appeared to rest.

*Tail skin of adult hairless mice* differed strikingly from other observed skin in appearance. When studied with the dissecting microscope, the arrangement of the corneal plates was suggestive of that of stones in a wall. These rounded-edged plates (Fig. 2A) were separated by wide annular grooves running around the tail (B), and by more narrow short longitudinal grooves (C) which extended only from one annular groove to the next. The plates did not overlap as in tail skin of haired (C3H) mice, but appeared to be an integral part of the *stratum corneum* making a relatively smooth continuous surface. Follicular orifices (D), mostly without projecting hair shafts, were easily recognized as small round indentations in groups of 3 in the annular furrows.

These orifices were either patent, covered by *stratum corneum,* or plugged by a remnant of a hair shaft or by a mass of sebum. The orifices of each trio were separated by about the widest diameter of a cercarial body; trios, outer orifice to outer orifice, were about 0.5 mm apart. The corneal plates of hairless mouse tail were not so thick and hard to the touch as those of C3H mice, but they appeared to be equally impervious to cercarial penetration.

Observed entry sites are listed in Table III. Three of the penetrants by follicular orifices entered at the edge of the water drop; 7 pushed under the *stratum corneum* against small disoriented hair shafts projecting about 1 mm above the skin surface.

Cercarial failures were numerous (Table II), and 2 cercariae returned to the surface. Both had entered against hair shafts but had then changed direction to break through the *stratum corneum* after several minutes in the skin, leaving U-shaped tunnels. Though the preacetabular glands and ducts were still filled with purpurin-stained contents, the cercariae were apparently exhausted. Both made further attempts at entry at adjacent non-patent follicular orifices but did not succeed, and soon became motionless.

One unsuccessful entry attempt did not always mean permanent failure, however. Another cercaria attempted entry through and around a non-patent follicular orifice for 6 minutes before moving to a patent orifice where entry took 4 minutes.

Exploratory and entry times are shown in Table IV. Migration beneath the skin surface could not be observed because of the opacity of the surface layers.

*Tail skin of adult haired (C3H) mice* differed in appearance from that of hairless mice in several ways which are relevant to the problem of cercarial penetration. Instead of the usual continuous corneal surface, hard corneal plates (Fig. 3A) covered haired mouse tails. The plates were arranged in series with the distal edges of each series overhanging the proximal edges of the succeeding series. Plates could thus be lifted off by inserting a needle under the overhanging edge in the annular grooves (B) which ringed the tail. Follicular orifices (C) occurred in 3's in these annular grooves. Sturdy, spindle-shaped hairs (D) about 1 mm long projected regularly from the follicular orifices under the trailing edge of the corneal plates. Short longitudinal furrows (E) perpendicular to the annular grooves separated the plates of each series.

Cercariae were much more successful in entering this skin than that of hairless mouse tails (Table II). When placed on tail skin of a haired mouse they either looped down the first hair with which they made contact, dropped gradually from one hair to another, or sank directly to the skin surface between hairs. There they either attempted entry at the first site touched or looped along briefly to a satisfactory site. Both the percentage of failures in penetration (Table II) and the average time spent in exploration on this skin (Table IV) were, except for human skin, the lowest recorded in this study. Entry sites and average entry times are shown in Tables III and IV.

The site of choice was through the follicular orifices beside the hair shaft, but rarely a cercaria entered under a hair at the point of last contact of the hair with the corneal plate. Since hairs projected at an acute angle with the tail surface, this point was along the hair shaft at some distance from its exsertion. Cercariae entering either by way of a follicular orifice or under a hair at its most distal point of contact with the corneal plate surface, did so in such close contact with the hair shaft that the tip shook with each probe of the schistosome. Schistosomula could not be followed under the surface of this skin. One penetrant came onto the surface again after having been in the skin for 2 minutes, and another bored under a fibrous strip of *stratum corneum* which loosened to leave it still above the skin surface.

*Body skin of 1- to 2-day old mice* had no patent follicular orifices, and no hair shafts had broken through (Fig. 4). Young mouse skin was more transparent and reddish, and smoother than adult skin. It was finely traced by long furrows (A) which intersected frequently to mark off triangular or hexagonal skin areas. In the center of each of these, the non-patent follicular orifices were slightly elevated on follicular mounds (B). Short, shallow secondary wrinkles (C) furrowed the entire surface.

Cercarial entry into this skin differed from that described for ear skin in several ways which may reflect the absence of patent follicular orifices. Fewer parasites were able to enter (Table II); and those which succeeded did so almost exclusively by way of wrinkle walls, either those at the bases of follicular eminences or the secondary wrinkles (Table III). Twelve percent of the penetrating cercariae followed a route of entry already opened by a schistosome which had entered previously. Exploratory and entry times did not differ markedly from those in ear skin (Table IV).

Twenty of the 48 entrances at wrinkle walls were at the edge of the drop of

water. Seven of the 31 unsuccessful attempts were also made in this area. This suggests that perhaps in relatively smooth skin which offers so few surface irregularities, the edge of the drop of water may provide an angle for the beginning of penetration (Faust *et al,* 1934).

Failures to enter appeared to be correlated with the same behavior as in ear skin. The barrier effect was intensified when the skin was tightly stretched over the cork so the deep wrinkles were eliminated. A few entered only to return to the surface. Usually these attempted entry again at another site, some successfully, some unsuccessfully.

Of possible significance for future work was the observation that 3 mice of one litter had skin which, though unstretched, was not entered by any of the applied cercariae. Skin excised from a fourth mouse of the same litter sacrificed 24 hours later was entered by 6 of the 10 cercariae applied. Of these, 1 died at the entry site, and 1 came back to the surface. Four of the 10 failed in spite of energetic entry attempts. In the latter instance, of course, the mouse was older by a day, and the pool of cercariae was collected from a different group of snails.

Some penetrants delayed detachment of the tail, so that this appendage was pulled completely into the migration tunnel. Tails were sometimes retained so long that their continued quivering may have been an aid in locomotion in the skin. Locomotion was unusually fast. One schistosomula traveled 0.9 mm in 5 minutes. Otherwise, migration in this skin was similar to that in ear skin of hairless mice.

*Body skin of 4- to 5-day old mice* differed from that of 1- to 2-day old mice only in that the wrinkles had deepened slightly, and many follicular orifices had fine hairs, about 0.5 mm long by 10 microns in diameter at the base, projecting beyond the skin surface (Fig. 5). Cercarial activity on the 2 baby mouse skin types may be compared in Tables II to IV. Observations similar to those described on skin of 1- to 2-day old mice were recorded with reference to the resurfacing of schistosomula, to the detachment of tails, and to entrance at the edge of the water drop. A small number of penetrants made use of hair shafts by pushing against them directly into the *stratum corneum* at the hair-skin angle, the acute angle produced by the slant of the hair with reference to the skin surface.

The proportion of failures to enter was lower than into the younger mouse skin (Table II) but this can be accounted for largely by the fact that no completely resistant skins were encountered in the older group. Movement of schistosomula within this skin was similar to that already described. Locomotion was slower than in the younger mouse skin, but was extremely variable. One schistosomula took only 8 minutes to travel 300 microns; two, 20 minutes; and one, 50 minutes.

*Body skin of adult hamsters* (Fig. 6) afforded clear observation of cercarial behavior on an unshaved haired skin. Many hairs either shared a common follicular orifice or emerged in close proximity as a bundle. Wide spaces separated these hair groups and vision into areas between was unimpeded, since there was no mat of fine hair on the skin surface. As the hairs emerged from the skin, the tissue adhered tightly to them. A skin fold which appeared as a long corneal ridge was usually thrown up over the follicular orifices.

The skin presented a roughened appearance produced by numerous small elevated areas outlined by shallow wrinkles which stretched in long straight lines. The absence of abrupt individual eminences around the follicular orifices and of deep

frequently intersecting wrinkles reduced the bending and separating of the layers of the *stratum corneum*. Masses of sebum (Fig. 6A) adhering closely to the skin, the roughened condition of the surface, and numerous acute hair-skin angles (B), however, provided the surface irregularities which seem to be necessary for cercariae to begin entry.

Entry sites, therefore, differed somewhat in skin of hamsters and adult hairless mice (Table III). Penetrants by follicular orifices entered either against the hair shaft itself or between the hairs of a group. Some cercariae curled around the base of the hair, attaching orally so close against the shaft that the whole hair moved as the parasite progressed into the skin.

Migrants within skin were followed satisfactorily. Locomotion was variable as yielding or resistant areas were encountered. Top speed was 347 microns in 3.5 minutes, but after this period the migrant made no further progress during the observational period of an hour. Two schistosomula returned to the surface. This seems to be a not uncommon occurrence, without relation to source of skin or to length of time after skin biopsy.

*Body skin of 5-day old rats* was chosen for two reasons to represent the rat, a host resistant to *S. mansoni*. First, unshaved body skin of adult rats could not be adapted to the presently-used techniques on account of the mat of fine hair in contact with the skin; and second, rat tail skin did not permit formation of drops of water but allowed immediate spread of the cercarial suspension over the skin surface.

Young rat skin was irregularly furrowed with both deep and shallow intersecting wrinkles. Fine hairs about 2 mm long were regularly spaced at intervals of about 1 mm. This skin had lost the red translucent appearance of younger rat skin and was pink and thickened. Except for a thicker layer of *stratum corneum*, it was similar in appearance to that of 4- or 5-day old mice.

Cercariae penetrated only occasionally (Table II) and with difficulty. Exploratory and entry times were among the longest for any skin (Table IV). Of the 66 percent which failed, many made no serious entry attempt, swimming or lying quietly on the skin. Some attempted entry to the extent that they assumed the appearance of a penetrating schistosome and even buried the oral end in the *stratum corneum*, but did not penetrate farther and gradually ceased movement. These remained partly in and partly out. Their preacetabular glands paled quickly so that within 6 or 7 minutes no stained material could be seen. Other cercariae attached to the skin surface indirectly, tethered by ropy mucoid secretion from the acetabular gland complex. Such tethering was not observed on any other skin. Since this material was pink when the glands were stained vitally with purpurin, and purple when it was secreted into a dilute solution of lithium carmine (Vogel, 1932), both pre- and post-acetabular glands appeared to be involved in its production. A similar reaction was observed in cercariae on a microscope slide in a drop of serum from infected hosts (Stirewalt and Evans, 1955) or in a drop of physiological saline. It may be a general reaction to unfavorable conditions, and, if so, suggests that perhaps rat sebum is an inimical factor. Rat tail sebum has been observed to differ from that of other skin in that water in contact with it does not form drops but spreads. Perhaps it differs also in its effect on cercariae. Only one penetrant entered at the edge of the water drop. Two entered against unsuccessful parasites stuck in their entry sites. In the latter case, this region may have

been softened by glandular material secreted during the earlier entry attempt. These observations were made on relatively few penetrants since the percentage of cercariae entering was low.

Migrants beneath the *stratum corneum* were difficult to follow because of the thickness of this layer and because of the almost complete loss of color of the cercarial glands during entry. The progress of 3 schistosomula was followed during which time the distances covered were: 450 microns in 15 minutes, 600 microns in 30 minutes, and 300 microns in 6 minutes. One of the eleven schistosomula returned to the surface after 3 minutes in the skin.

*Adult monkey skin* was a heavy, wrinkled chest skin with a thick *stratum corneum*. Long hairs of large diameter projected irregularly from the deepest primary wrinkles. Secondary wrinkles furrowed the areas enclosed by the deeper primary wrinkles.

Observation of cercarial entry was difficult because of the poor light-transmitting and -reflecting quality of the skin. Only the dissecting microscope and reflected light could be used.

Cercarial behavior on this skin differed from that on ear skin of mice only in the details listed in Tables II to IV. Most cercariae entered monkey skin and the time spent in exploration and in entry was about average. Only in body skin of 5-day old rats did the number entering by used entry sites approach that in monkey skin.

Migration could not be followed because of the thickness of the *stratum corneum*.

*Adult human skin* was from 3 sources. Skin from the dorsum of a great toe (Fig. 7) had been removed from the body 24 hours earlier, and had been frozen and thawed. It was furrowed with deep broad primary wrinkles (Fig. 7A) enclosing relatively large triangular or rectangular areas (B) and shallow narrow secondary wrinkles (C) which surrounded small irregularly-shaped elevations (D). Several hairs of large diameter were exserted from the deep primary wrinkles.

The abdominal skin was taken from a cadaver 18 hours after death. The surface was smoother than that of the toe skin, but the pattern of wrinkling was similar. No hairs were present and no openings into the skin were observed with the dissecting microscope.

The skin of the finger tip and of the dorsum of the basal joint of the middle finger was observed *in situ* on a normal individual. Finger tip skin was ridged in the pattern familiar in fingerprints, with the openings of the sweat glands regularly placed on the ridge tops. Skin of the dorsum of the basal joint of the middle finger showed only a few shallow wrinkles enclosing relatively large areas of almost smooth surface. Several singly exserted hairs protruding from depressions in the wrinkles were the only breaks observed in the surface.

The cercarial penetration pattern was similar to that on other skins. It was also similar in all observed respects on excised skin and on that *in situ*. Penetration was good (Table II) and entry sites were, without exception, in wrinkles (Table III). Exploration and entry times are given in Table IV.

## DISCUSSION

The observed cercarial behavior pattern agrees in general in the features discussed with those of other authors, the most recent detailed descriptions being those of Gordon and Griffiths (1951) and Standen (1953). Differences of detail such

as the choice of entry sites, the movement of the tail in penetration, and the persistence of the raised lip around the entry pore are, without doubt, related to characteristics peculiar to different skins, to the individual reactions of cercariae to skin, to techniques employed, such as pre-exposure skin treatment, and especially the number of parasites entering at the same time.

Several points concerning the reference list in Table I require special comment. Fujinami and Nakamura (1909) mentioned by Standen (1953) were not listed in Table I because they did not take a stand on the question of the point of entry of cercariae into skin, but were concerned only with the problem of infection via skin as opposed to mucous membranes of mouth or nose. Miyagawa (1912), Koppisch (1937), Pinto and Almeida (1948), and Gordon and Griffiths (1951) recorded entry not only through *stratum corneum* as listed by Standen but also by hair follicles, though they differed as to which was the more frequently used point of entry. Cort's 1921 description was of a cercarial dermatitis in which each papule centered in a hair follicle. Only by inference, then, was this listed as follicular penetration. Finally, follicular penetration described by Watarai (1936) was along downy feathers instead of hairs.

It is obvious that the choice of entry sites by cercariae varied under different conditions and in dissimilar skins (Table III). Skin differences are reflected in the ratio of penetrants to failures, in the choice of entry sites, and in the average time spent in exploration and actual entry (Tables II, III, and IV). Examination of Table II shows that 4 groups of host skin may be separated on the basis of the proportion of cercariae entering: the first 3 types of skin entered by over 85%; the next four, by 73–78%; the next two, by 62–65%; and the last, by less than 50%.

Reference to Table IV reveals little correlation between the proportion of cercariae entering a skin and the average length of time of exploration or entry except for the skin of 5-day old rats. In this skin the *stratum corneum* was thick, though deeply fissured, and the fine hairs which had broken through the surface were apparently of such small diameter that they were not suitable entry sites. Doubtless the resistance of the *stratum corneum* in terms of smoothness, toughness and thickness, the number and distribution of entry sites, the physiological condition of the cercaria and choice of sites attacked are all variables of importance.

A listing of entry sites according to frequency of use by the 383 penetrants places wrinkle walls first with 158 cercariae, and follicular orifices second with 146 cercariae. Fifty-six entered at the angle made by some material with the skin, a mass of sebum, a skin scale, the curved edge of the water drop, a hair lying on the skin, or the distal hair-skin angle. Fourteen used the entry site of a previous penetrant, either passing through the patent lesion or along a cercarial tail left in the lesion. Nine entered the tail skin of mice through the annular groove.

Different skin areas of the same host vary also as regards penetration. Both ear and body skins of hairless mice have follicular orifices similar in number, distribution and condition but body skin is much more deeply and frequently furrowed than ear. By contrast, the latter appears stretched. The percentage of penetrants is roughly the same into both skins, but the route of choice leads through follicular orifices in ear skin and through wrinkles in body skin. Experimental stretching of deeply wrinkled skin reduced the number of cercariae entering through wrinkles with the result that fewer cercariae penetrated stretched skin without many follic-

ular orifices. Both ear and body skins are penetrated by a large proportion of cercariae, but tail skin from the hairless mouse is less readily penetrated. This probably reflects the fact that this skin covered with a hard smooth *stratum corneum* offers only follicular entrance sites, most of which are overgrown with *stratum corneum*. It may be recalled that tail skin of adult haired (C3H) mice, studded regularly with many hairs of large diameter, ranks at the top of the list of skins ordered by tabulated percentage penetration.

No specific chemotactic factor influential on cercarial entry was identified, though the fact that when cercariae were applied to skin in a group they seemed drawn together to enter, suggests such a factor. Cercariae responded, as described earlier, to skin as to any surface which is presented, even a piece of wood or the surface of a microscope slide. The fact that some cercariae seemed unaware of skin was related to individual parasite variation or impotence and not to the skin surface itself.

Because of the different techniques employed by those who have timed cercarial penetration, comparison of data is not particularly informative. According to Hitchcock (1949), the average time cercariae spent from their application to the clipped abdominal skin of the hamster to their disappearance into it was 4.3 minutes, (range, 2 to 15 minutes); in ear skin of mice it was 3.6 minutes (range, 2.5 to 8 minutes). This was much faster entry than that recorded in Table IV, probably because the former periods were for cercariae applied in groups while the latter were for cercariae entering singly. It has been noted during these studies that when cercariae are applied in groups, several usually "collaborate" on one entry area to the end that a greater proportion enter and the entry period is shorter. Such comparison was made by Griffiths (1953).

Similarly, the data of Pan *et al* (1954) cannot be informatively compared with those presented here. The host skin was clipped and shaved, the cercariae were *S. japonicum*, and the skin was quickly dried after exposure. Under these conditions some cercariae entered and developed in mice after 10 seconds on the wet skin and in hamsters after 30 seconds. Heavy infections developed in both animals after 3-minute exposures. As the authors suggest, entry was not observed and the cercariae may not have completed penetration during the period recorded but only have gained access to a protected crevice in which drying would be slow and entry could thus be completed. Unobserved exposures of haired albino mice by tail to *S. mansoni* cercariae for 15, 30, 45 and 60 minutes resulted in penetration of about 78 percent after 15 and 30 minutes and of about 90 percent after 45 and 60 minutes of exposure (Olivier and Stirewalt, 1952). Whether this discrepancy can be referred to species differences or whether it only reflects procedural variations remains to be determined. These facts all emphasize, however, that the conditions of the experiment influence markedly the results obtained.

Disagreements of this nature probably cannot be ascribed to differences between skin on and off the host. Part of our observations were made on excised skin and part on skin *in situ*. No observable variations in cercarial behavior were noted under the two conditions, though these findings do not agree with Leiper's (1915) statement that he saw cercariae enter the skin of dead mice only rarely. Even the return of penetrants to the surface, apparently not reported by others, was noted in skin under both conditions. This phenomenon occurred most often when entry

as the choice of entry sites, the movement of the tail in penetration, and the persistence of the raised lip around the entry pore are, without doubt, related to characteristics peculiar to different skins, to the individual reactions of cercariae to skin, to techniques employed, such as pre-exposure skin treatment, and especially the number of parasites entering at the same time.

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Reference to Table IV reveals little correlation between the proportion of cercariae entering a skin and the average length of time of exploration or entry except for the skin of 5-day old rats. In this skin the *stratum corneum* was thick, though deeply fissured, and the fine hairs which had broken through the surface were apparently of such small diameter that they were not suitable entry sites. Doubtless the resistance of the *stratum corneum* in terms of smoothness, toughness and thickness, the number and distribution of entry sites, the physiological condition of the cercaria and choice of sites attacked are all variables of importance.

A listing of entry sites according to frequency of use by the 383 penetrants places wrinkle walls first with 158 cercariae, and follicular orifices second with 146 cercariae. Fifty-six entered at the angle made by some material with the skin, a mass of sebum, a skin scale, the curved edge of the water drop, a hair lying on the skin, or the distal hair-skin angle. Fourteen used the entry site of a previous penetrant, either passing through the patent lesion or along a cercarial tail left in the lesion. Nine entered the tail skin of mice through the annular groove.

Different skin areas of the same host vary also as regards penetration. Both ear and body skins of hairless mice have follicular orifices similar in number, distribution and condition but body skin is much more deeply and frequently furrowed than ear. By contrast, the latter appears stretched. The percentage of penetrants is roughly the same into both skins, but the route of choice leads through follicular orifices in ear skin and through wrinkles in body skin. Experimental stretching of deeply wrinkled skin reduced the number of cercariae entering through wrinkles with the result that fewer cercariae penetrated stretched skin without many follic-

ular orifices. Both ear and body skins are penetrated by a large proportion of cercariae, but tail skin from the hairless mouse is less readily penetrated. This probably reflects the fact that this skin covered with a hard smooth *stratum corneum* offers only follicular entrance sites, most of which are overgrown with *stratum corneum*. It may be recalled that tail skin of adult haired (C3H) mice, studded regularly with many hairs of large diameter, ranks at the top of the list of skins ordered by tabulated percentage penetration.

No specific chemotactic factor influential on cercarial entry was identified, though the fact that when cercariae were applied to skin in a group they seemed drawn together to enter, suggests such a factor. Cercariae responded, as described earlier, to skin as to any surface which is presented, even a piece of wood or the surface of a microscope slide. The fact that some cercariae seemed unaware of skin was related to individual parasite variation or impotence and not to the skin surface itself.

Because of the different techniques employed by those who have timed cercarial penetration, comparison of data is not particularly informative. According to Hitchcock (1949), the average time cercariae spent from their application to the clipped abdominal skin of the hamster to their disappearance into it was 4.3 minutes, (range, 2 to 15 minutes); in ear skin of mice it was 3.6 minutes (range, 2.5 to 8 minutes). This was much faster entry than that recorded in Table IV, probably because the former periods were for cercariae applied in groups while the latter were for cercariae entering singly. It has been noted during these studies that when cercariae are applied in groups, several usually "collaborate" on one entry area to the end that a greater proportion enter and the entry period is shorter. Such comparison was made by Griffiths (1953).

Similarly, the data of Pan *et al* (1954) cannot be informatively compared with those presented here. The host skin was clipped and shaved, the cercariae were *S. japonicum*, and the skin was quickly dried after exposure. Under these conditions some cercariae entered and developed in mice after 10 seconds on the wet skin and in hamsters after 30 seconds. Heavy infections developed in both animals after 3-minute exposures. As the authors suggest, entry was not observed and the cercariae may not have completed penetration during the period recorded but only have gained access to a protected crevice in which drying would be slow and entry could thus be completed. Unobserved exposures of haired albino mice by tail to *S. mansoni* cercariae for 15, 30, 45 and 60 minutes resulted in penetration of about 78 percent after 15 and 30 minutes and of about 90 percent after 45 and 60 minutes of exposure (Olivier and Stirewalt, 1952). Whether this discrepancy can be referred to species differences or whether it only reflects procedural variations remains to be determined. These facts all emphasize, however, that the conditions of the experiment influence markedly the results obtained.

Disagreements of this nature probably cannot be ascribed to differences between skin on and off the host. Part of our observations were made on excised skin and part on skin *in situ*. No observable variations in cercarial behavior were noted under the two conditions, though these findings do not agree with Leiper's (1915) statement that he saw cercariae enter the skin of dead mice only rarely. Even the return of penetrants to the surface, apparently not reported by others, was noted in skin under both conditions. This phenomenon occurred most often when entry

had begun under a skin scale or a mass of sebum. It was not related to the length of time after skin biopsy.

Migration through peripheral tissues, as studied in serial sections of penetrated skin, will be the subject of another paper in this series. Direct observation stressed the use of the corneal-Malpighian juncture as a route of migration, the combination of constant oral exploration with secretion from the acetabular glands as the means of formation of the migration tunnel, and the contrast of the liquid contents of the tunnel with the gel consistency of its limiting walls.

## SUMMARY

Behavior of cercariae of *Schistosoma mansoni* was observed and described on 10 skin types from 5 different definitive hosts: mouse, rat, hamster, monkey and man. The percentages of cercariae penetrating, entry sites, and exploration and entry times were recorded. Early migration was followed in those skins which afforded the requisite visibility.

Adult human and monkey skin and tail skin of haired mice were penetrated by over 85% of the applied cercariae; ear and body skin of adult hairless mice, body skin of adult hamsters and of 4- to 5-day old mice, by 73 to 78%; body skin of 1- to 2-day old mice and tail skin of adult hairless mice, by 65 and 62%, respectively; and body skin of 5-day old rats, by 34%.

Breaks, bends, angles and frank openings in the *stratum corneum* all featured in cercarial penetration. Entry sites of choice varied with the skin from wrinkle walls in human skin to hair follicles in tail skin of haired mice.

Times spent in exploration were shortest in skin with numerous, well-distributed, favorable entry sites, a minute or less on human skin; and longest, averaging over 4 minutes, on body skin of 5-day old rats.

Actual entry times were about 3 minutes into body skin of 1- or 2-day old mice and ear and body skin of adult hairless mice, and about 7 minutes into body skin of 5-day old rats, tail skin of adult hairless mice, body skin of adult hamsters, and adult human skin.

Patterns of penetration and migration were similar on all the skins. Penetration involved orientation to the entry site, close oral attachment, great muscular activity and copious glandular secretion. Migration was accomplished by a combination of muscular and glandular activity with constant oral exploration until yielding intercellular avenues were encountered.

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## EXPLANATION OF PLATE

FIGURE 1. Abdominal skin of adult hairless mouse (×20). A. Follicular orifice topping conical eminence; B. Primary wrinkle.

FIGURE 2. Tail skin of adult hairless mouse (×24). A. Corneal plate; B. Annular groove; C. Longitudinal groove; D. Follicular orifices.

FIGURE 3. Tail skin of adult haired (C3H) mouse (×24). A. Corneal plate; B. Annular groove; C. Follicular orifices; D. Hairs; E. Longitudinal furrow.

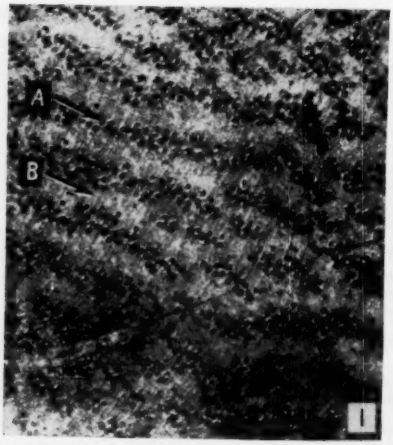
FIGURE 4. Abdominal skin of 1-day old mouse (×20). A. Primary wrinkle; B. Follicular mounds; C. Secondary wrinkle.

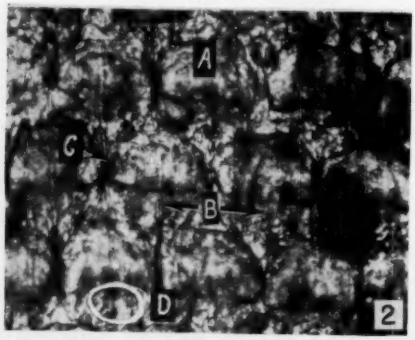
FIGURE 5. Abdominal skin of 4- to 5-day old mouse (×24).

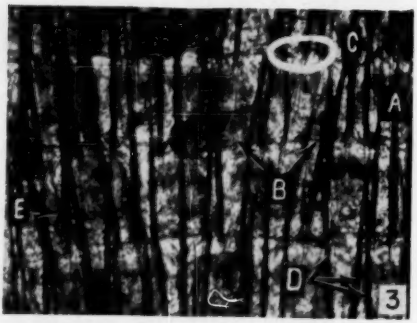
FIGURE 6. Abdominal skin of adult hamster (×24). A. Masses of sebum; B. Acute hair-skin angle.

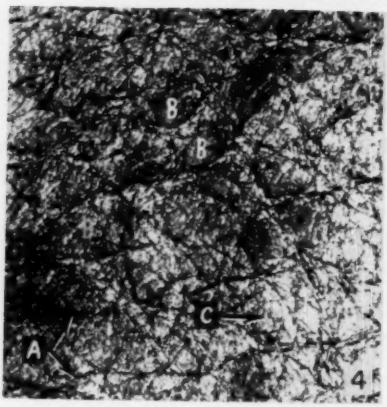
FIGURE 7. Skin from dorsum of great toe of adult man. (×28). A. Primary wrinkle; B. Elevated area; C. Secondary wrinkle; D. Small elevation.

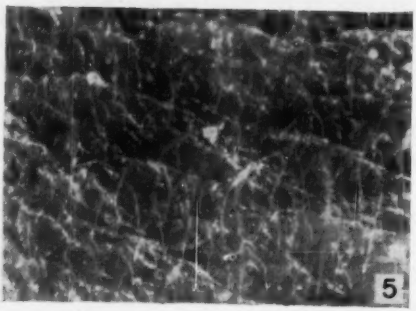
PLATE I

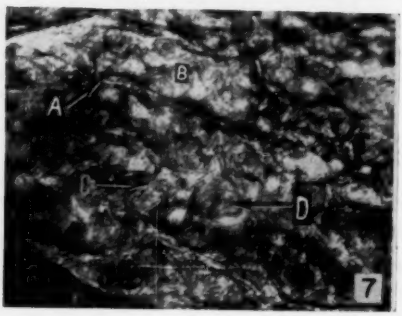




# COLD CULTURE

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Flagellates belonging to the genera *Cercomonas* and *Copromonas* (Figs. 1 and 2) were found in fresh samples of 8 cattle feces sent to the author in November, 1955. The former averaged $2.5 \times 5.4$ microns and possessed a long trailing flagellum and an extremely short anterior flagellum. The latter averaged $8.4 \times 4$ microns and possessed a single, long anterior flagellum. The material was examined with a phase-contrast microscope, with and without neutral red vital stain. Permanent slides were prepared using Heidenhain's iron-hematoxylin stain. All samples were placed in small plastic vials, covered with tight-fitting caps, and stored in a refrigerator kept at 4° C.

One month later motile flagellates were present in greater numbers than they were when first examined. Also, a few motile amoebae (Fig. 3) were found. These amoebae averaged 39 microns in diameter and produced large, rounded pseudopodia. After another week in cold storage, one sample was found to contain, in addition to those already observed, another species of flagellate. The new one averaged 4 microns in length and possessed 2 anterior flagella. It was apparently *Monas communis*, found by Becker and Talbott in 1927 from cattle rumen contents (Fig. 4). By 19 December the cold culture was swarming with the various flagellates and numerous motile amoebae. The latter appeared to belong to the genus *Acanthamoeba* or *Vahlkampfia* although they seemed to be unlike *Vahlkampfia lobospinosa* also reported by Becker and Talbott (1927) from the rumen. Amoebae from the cold culture possessed a large contractile vacuole. Usually there was only 1 pseudopodium which was broad and clear and frequently formed in an almost explosive manner. The nucleus possessed a large endosome. Within the thin nuclear membrane there usually could be seen a lightly-staining irregular ring of material. The cysts were more variable in appearance. There was a fine gradation between the *Entamoeba bovis* type and the *Vahlkampfia* variety. A special study is being made of these amoebae. Motile flagellates were most abundant just under the edge of the coverslip and around the periphery of air spaces. Many individuals were dividing. During subsequent weeks there was a gradual decline in numbers of protozoa but they have persisted in some samples for the past 5 months.

The experiment with cold culture was repeated with fecal samples from cattle in another locality. When kept under refrigeration there occurred an increase in numbers and species of motile flagellates and motile amoebae, even in samples in which there was at first no evidence of motile forms. One sample was selected for special observation and showed a typical sequence of events. During the first few weeks there was a gradual change in the protozoan picture with a decrease in numbers of the small flagellates but an increase of the larger variety. At the end of 2 weeks the sample teemed with enormous numbers of highly motile bacteria. A little mold and a few yeast spores could also be seen. Three months after collec-

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tion, 1 to 10 motile amoebae appeared in almost every field of view as seen with the oil immersion objective. These amoebae seemed more sluggish than those from the first experiment.

Feces of pigs and sheep were treated in the same manner. The sheep material yielded many yeast and mold spores but no motile protozoa. The pig samples at first showed only *Balantidium* (some in tremendous numbers). After 18 days at 4° C there appeared a moderate number of *Cercomonas* and *Copromonas* of the same species as found in cattle. The flagellates increased in numbers and thrived for several months but gradually died out, being replaced by the packet-forming bacterium *Sarcina*.

All specimens were maintained in cold culture with no attention except the addition of 2 or 3 drops of saline about once a month. If the samples were kept more than just moist with only a few drops of fluid at the bottom of the vial, the protozoa diminished in numbers. A few vials developed a little mold. There seemed to be a direct correlation between the abundance of motile protozoa and motile bacteria.

Care was taken not to contaminate the fecal specimens with soil. Some samples were taken directly from the anus of cattle, others were taken from the top of fresh material dropped on newly scrubbed concrete floors. Soil samples, however, are being kept under various conditions of humidity and temperature and are under examination for protozoa. So far, only a few unidentifiable cysts have been found. None has excysted in the laboratory at room temperatures or in the refrigerator. Culturing techniques with various media kept at 37° C are also being carried on, so far without success. Similar experiments with human intestinal parasites have been started.

It seems likely that all the protozoa which thrive in cold culture are coprozoic forms. *Cercomonas, Copromonas,* and *Vahlkampfia* have been found in other hosts. Reports of cattle amoebae indicate that almost all of them belong to the genus *Entamoeba* (Noble and Noble, 1952). These reports are based almost exclusively on examinations of cysts. It is probable that the *Vahlkampfia* type is much more common than supposed. Possibly most amoebic cysts which have been identified as *Entamoebae* in domestic mammals are really varieties of *Vahlkampfia* or *Hartmanella*. Certainly it is practically impossible to draw a sharp line of distinction by simply observing cysts in fecal smears.

Cold culture probably does not reveal anything that other types of culture would not show. Excystation at room temperatures undoubtedly would occur if the material were kept free from mold, desiccation and decay. Cold culture has a big advantage in its remarkable simplicity and ease of maintenance. The significant question is why some intestinal protozoa will excyst and remain active for months at 4° C or some other low temperature.

## SUMMARY

Fresh cattle feces was found to contain motile flagellates belonging to the genera *Cercomonas* and *Copromonas*. Amoebic cysts and some unidentifiable cysts were also present. This feces was kept in a refrigerator at 4° C for 5 months. A few drops of saline were added every few weeks to prevent drying. During this time one more species of flagellate was observed (*Monas communis*) and many

of the amoebae excysted. These motile amoebae were of the *Vahlkampfia* type. There was no evidence that *Entamoeba bovis* excysted. The material became a cold culture in which the protozoa thrived and at least some of the flagellates reproduced. Pig feces was similarly treated. The same species of *Cercomonas* and *Copromonas* appeared but no motile amoebae were found.


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DESCRIPTION OF FIGURES

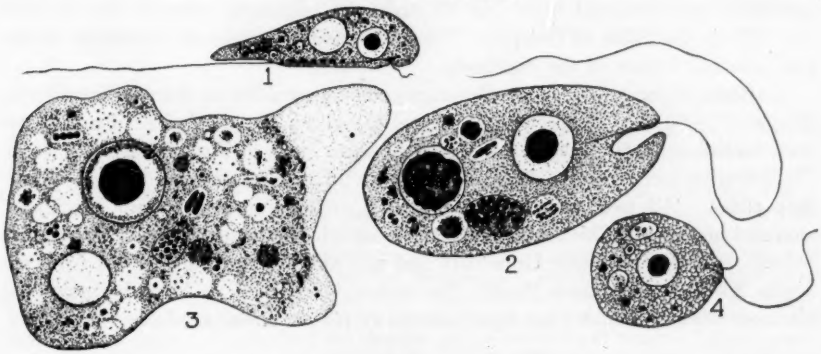


Figure 1. *Cercomonas* sp. (×5000) showing long trailing flagellum with associated granules, short anterior flagellum, large vacuole and typical nucleus.

Figure 2. *Copromonas* sp. (×6000) showing numerous cytoplasmic inclusions, cytostome and long, single anterior flagellum.

Figure 3. *Vahlkampfia* sp. (×1600) showing clear broad pseudopodia, contractile vacuole and light-staining ring around the large endosome.

Figure 4. *Monas communis* (×5000) showing the two anterior flagella, one short and one long, some cytoplasmic inclusions and the typical flagellate nucleus.

## RESEARCH NOTE

### THE BITING LOUSE *PITRUFQUENIA COYPUS* MARELLI ON NUTRIA IN LOUISIANA

During a survey of the parasites of nutria, otter, and mink in Louisiana, in collaboration with the Fur and Refuge Division of the Louisiana Wildlife and Fisheries Commission, 1954–55, Mr. John F. Schacher found one species of ectoparasite on feral nurtia (*Myopotamus coypus*). It was identified as *Pitrufquenia coypus* Marelli (Mallophaga, Gyropidae, Gliricolinae), a South American species which is evidently a specific parasite of nutria, introduced into the United States with its host. Specimens agree with descriptions of the species given by F. L. Werneck (1936, Mem. Inst. Oswaldo Cruz **31**: 391–589) and R. B. Freeman (1946, Ent. Monthly Mag. **82**: 226–227). The lice (16 males, 18 females, and 51 nymphs) were found on the snout of an immature female nutria (the only one examined for ectoparasites) collected in the Marsh Island Refuge, 30 miles south of New Iberia, Louisiana, on January 31, 1955. To our knowledge, this is the first record of this mallophagan in the United States. Freeman (1946) states that it was originally described in 1932 from a Coypu farm in Chile and was subsequently reported from 2 farms in Argentina and from a wild nutria in England.—ALBERT MILLER, *Department of Tropical Medicine and Public Health, School of Medicine, Tulane University, New Orleans, La.*

# TWO NEW HYMENOLEPIDID CESTODES FROM MEXICAN BIRDS WITH OBSERVATIONS ON *HYMENOLEPIS CROCETHIAE* WEBSTER, 1947

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The Ohio State University

A group of cestodes collected in southern Mexico yielded several worms which could be assigned to the genus *Hymenolepis* and of these, 2 appeared to be new species. Three complete specimens were obtained from a charadriiform bird (collared plover) collected on September 13, 1954 near Salina Cruz, Oaxaca and 14 specimens were obtained from 3 of 10 Acorn Woodpeckers collected during 1954 and 1955 in the State of Chiapas. The latter bird is a frequent inhabitant of the pine and oak forests in the highlands.

Cestodes of the genus *Hymenolepis* are common parasites of charadriiform birds. Hughes (1940) lists 43 species and several have been described since then. However, he lists only *H. crenata* and *H. rhodesiensis* from piciform birds.

Corrosive acetic fixative, Harris' haematoxylin, and terpineol were used to prepare slides. All new measurements are in millimeters taken from specimens mounted in damar. This study was carried out while the author was a Muellhaupt Scholar at The Ohio State University and was aided by a Grant-in-Aid from the Sigma Xi-RESA Research Fund. The author is indebted to Allen McIntosh for his cooperation in making the type material of *H. crocethiae* available for study.

*Hymenolepis chiapaensis* n. sp.
(Figs. 1, 3, 4, 6, 12–14, 16.)

*Diagnosis*: Hymenolepididae, with the characters of the genus. Strobila up to 50 long and 1.0 wide in gravid proglottids. Segments craspedote, broader than long in all instances, but ratio changing with age. Scolex 0.20 in diameter, with ellipsoidal suckers 0.12–0.13 in longitudinal diameter. 18 (rarely, 17) rostellar hooks, 0.051–0.059 long, arranged in single row. Rostellum withdrawn 0.18×0.093. Neck 0.16 long and 0.15 wide where strobilization begins. Genital fundaments appear when segments about 0.22 wide; primordia of genital ducts and testes become differentiated when proglottid about 0.3 wide. Longitudinal excretory vessels in gravid segments 0.022 to 0.029 (ventral) and 0.003 to 0.006 (dorsal). Transverse commissure in posterior half of proglottid connecting ventral longitudinal vessels. Genital pores unilateral, dextral. Genital atria deep, aspinose lined with heavy cuticle. Numerous cells surrounding atria, possibly of glandular function. Aspinose cirrus enters atrium dorsal and slightly anterior to vagina. Cirrus sac up to 0.028 in diameter; filled with prostate cells and small seminal vesicle. Wall of sac 0.003 thick. Ductus ejaculatorius without convolutions. External seminal vesicle up to 0.20 long in gravid proglottids, dorsal to seminal receptacle. External seminal vesicle starts to fill with sperm before ovary reaches full size; internal seminal vesicle fills short time thereafter. Vagina with heavy wall, probably nonmuscular, surrounded with loose matrix possessing numerous nuclei. Vagina enters atrium ventral and slightly posterior to cirrus; proximal part enlarged as seminal receptacle short distance from ovary. Cirrus sac, external seminal vesicle, vagina, and seminal receptacle all persist in displaced arrangement until segments break off strobila. Three testes dorsal in form of triangle, two aporal, one poral, ellipsoidal in shape reaching length of 0.12. Testes persist until uterus is about half-filled, then disintegrate slowly. Ovary irregularly, coarsely lobed, near mid-point of proglottid. Vitelline gland slightly aporal, rotund in shape, location variable, usually posterior and lateral to ovary. Ovary and vitelline gland disintegrate shortly after uterus starts to fill. Uterus a transverse tube of irregular diameter, later developing large diverticula. Eggs of typical hymenolepid type when alive, generally collapsed in mounted specimen. Embryonic hooks 0.0196 to 0.0182 long.

Received for publication, May 7, 1956.

*Host:* Acorn woodpecker (*Melanerpes formicivorus*).
*Locality:* Region of San Cristobal Las Casas, Chiapas, Mexico.
*Type specimen:* Holotype in U. S. National Museum Helminthological Collection No. 38162.

In the early development of the proglottid, the genital ducts are formed and the genital atrium is well-developed and easily seen, but does not open to the exterior. Opposite to the genital atrium is a thickened dimple in the cuticle and between the two is a "plug" of tissue with a profusion of nuclei.

*H. anceps* Linton, 1927, *H. sacciperium* Mayhew, 1925, *H. mergi* Yamaguti, 1940, and *H. kaiseris* Sharma, 1943, have been described as possessing 18 hooks, but can be differentiated from the species at hand by the shape and the size of the rostellar hooks. *H. tenius* (Clerc, 1906) Fuhrmann 1908 possesses hooks of a similar shape, but it differs by possessing fewer and shorter hooks.

*H. collariella* n. sp.
(Figs. 2, 5, 7, 8, 9, 10, 15.)

*Diagnosis:* Hymenolepedidae; with the characters of the genus. Moderate-sized cestodes up to 23 long and probably longer in well extended specimens. Strobila with segments broader than long, elongating with maturation. Gravid proglottids up to 0.95 wide. Scolex not easily lost, 0.19–0.22 in width at level of globose suckers which measure from 0.074 to 0.080 in diameter; spines not observed. Rostellum stout, armed with single row of 8 hooks, 0.012 to 0.014 long. Distance from scolex to region of strobilization 0.4. Genital fundaments appear at widely different times in different strobilae. Genital pores unilateral, dextral, located near midpoint of proglottid. Genital atria shallow, nonmuscular, without armature. Cirrus enters atrium anterior to vagina at almost same level. Cirrus sac very thin, almost completely filled with internal seminal vesicle in mature segments. Prostate cells not seen. Terminal part of cirrus heavy, appearing scleratized, yet flexible and eversible (0.12 long). Vagina heavy-walled distally, enlarging proximally to form large seminal receptacle. Neither armature nor sphincter observed. Cirrus sac, vagina, external seminal vesicle, and seminal receptacle all persist, being displaced anteriorly, in gravid proglottids. Three testes, of unequal size, up to 0.132 long, located in almost straight line (from dorsal aspect), persisting until uterus is about one-third filled. Ovary ventral, bilobed located centrally. Vitelline gland subrotund located at the center and posterior to ovary. Uterus saciform filling with eggs distally first. Hexacanths develop while still in uterus. Embryonic hooks 0.012 to 0.096 long.
*Host:* Collared plover (*Charadrius collaris*).
*Locality:* Near Salina Cruz, Oaxaca, Mexico.
*Type specimens:* Holotype in the U. S. National Museum Helminthological Collection, No. 38163.

*H. collariella* possesses hooks of somewhat similar shape to those found in *H. hamasigi* Yamaguti, 1940, (also from a shore bird) but can be differentiated from it by the possession of few hooks of shorter length.

A number of small cestodes were collected from a Sanderling (*Crocethia alba*) taken in the State of Oaxaca, Mexico. Study of these worms revealed that they were close to the description given for *Hymenolepis crocethiae* by Webster (1947). A careful study of the type material designated by Webster convinced me that the cestode from Mexico was *H. crocethiae*. The differences between Webster's description and the type material concerns the genital ducts. The vagina opens into a folded genital atrium ventral to the cirrus. The distal part of the vagina is funnel-shaped and provided with long slender spines. The part proximal to this is heavy-walled. The cirrus is short, eversible, and sparsely armed with short (less than 2 microns), rather stout spines. The glandular accessory pouches were not observed. The testes are situated in a triangle and the ovary is aporal.

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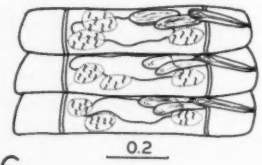


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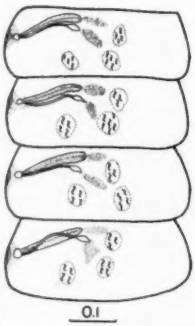


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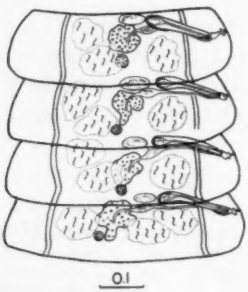


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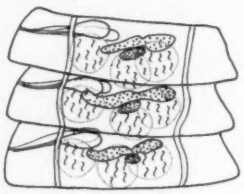


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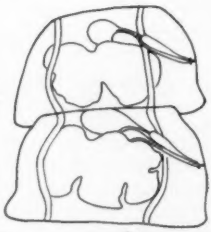


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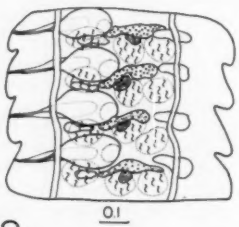


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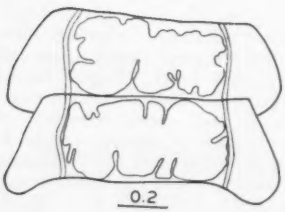


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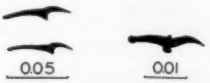


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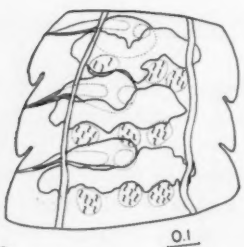


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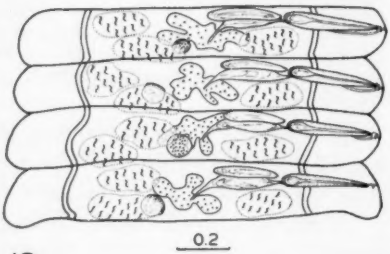


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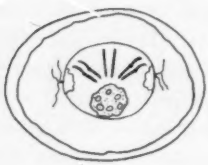

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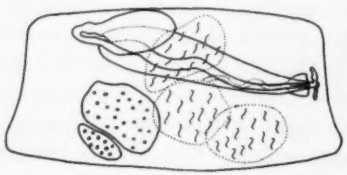

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EXPLANATION OF PLATE

(All figures drawn with the aid of a microprojector except numbers 1 and 11.)

1. Freehand sketch of living egg of *H. chiapaensis.*
2, 5, 7, 8, and 15. Proglottids of *H. collariella* at various stages of development.
3, 4, 6, 12, and 16. Proglottids of *H. chiapaensis* at various stages of development.
9. Embryonic hooks of *H. collariella.*
10. Rostellar hooks of *H. collariella.*
11. Freehand sketch of *H. crocethiae.*
13. Rostellar hooks of *H. chiapaensis.*
14. Embryonic hooks of *H. chiapaensis.*

## RESEARCH NOTE

### THE DAILY EGG PRODUCTION OF *ASCARIS SUUM* AND THE INABILITY OF LOW LEVELS OF AUREOMYCIN TO AFFECT EGG PRODUCTION AND EMBRYONATION

Three 120 lb. pigs infected with *Ascaris suum* were observed for 15 days. Daily worm egg counts were made by the direct Stoll dilution method and daily egg production of the ascarids was computed by multiplying eggs per gram of feces (EPG) by grams of feces excreted daily. The numbers below are averages for the 15-day period.

One pig, receiving Aureomycin at the rate of 10 mg per pound of feed, consumed about 66 mg of the antibiotic daily. The daily egg count was 13,709 EPG and daily egg production was calculated to be 11,600,000. Seven female ascarids were recovered at necropsy; thus each had produced about 1,600,000 eggs daily.

A second pig received Aureomycin at the rate of 50 mg per pound of feed and consumed about 284 mg of the antibiotic per day. The daily egg count for this pig was 1,309 EPG. Only 1 female was recovered at necropsy and she had produced about 1,600,000 eggs per day.

The third pig received no Aureomycin. It lost its infection on the 9th day. The daily egg count for the 9 days was 845 EPG and about 1,000,000 eggs were deposited daily. Although no females were recovered it is assumed that this pig harbored a single female until the ninth day.

The eggs were embryonated in a mixture of feces and granular charcoal maintained at 30°C. Embryonation was unaffected by the Aureomycin treatments. Ninety-six per cent of the eggs collected on the 7th day of the trial contained motile larvae after they had been incubated 13 days. Almost all of the eggs collected on the 15th day were embryonated after 16 days of incubation (based on observation of 50 eggs selected at random).

The females of *A. suum* in pigs of this trial produced about 1,400,000 eggs daily which is considerably higher than the 240,000 daily egg production of *A. lumbricoides* in man as reported by Brown and Cort (J. Parasitol. **14**: 88, 1927).—GEORGE W. KELLEY, JR. AND L. J. SMITH, *University of Nebraska, Lincoln, Department of Animal Pathology and Hygiene.* (Published with the approval of the Director as Paper No. 767, Journal Series, Nebraska Agricultural Experiment Station.)

# STUDIES ON MALARIA IN CHIMPANZEES
# I. THE ERYTHROCYTIC FORMS OF *PLASMODIUM REICHENOWI*

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During the course of various studies on the malaria parasites of chimpanzees, there occurred an opportunity for close and exact study of all erythrocytic stages of *P. reichenowi*. All asexual forms were seen in abundance in the peripheral blood contrary to the usual behavior of malignant tertian parasites, and a detailed description of the parasite, apart from its intrinsic value, may be of some use in the consideration of the related *P. falciparum*.

## MATERIALS AND METHODS

Among this Institute's colony of chimpanzees (*Pan troglodytes verus*), one animal, No. 5, was found to harbor a natural and mixed infection of *P. reichenowi* and *P. malariae* (*P. rodhaini*) with the former parasite predominating. 0.5 ml of blood was taken from No. 5 and inoculated intravenously into No. 1 chimpanzee. No. 1 had been treated 6 weeks previously with the full curative dosages of chloroquine and primaquine.

Twelve days after inoculation the spleen was removed from No. 1. During the subsequent malaria attacks, thick and thin blood smears were taken, stained with Giemsa stain and examined. Temperature records were kept for the few days of intense parasitemia. *P. reichenowi* was studied also as a natural infection in 7 whole chimpanzees.

On 2 occasions when scanty gametocytes of *P. reichenowi* were present in the blood of No. 1, *Anopheles gambiae* were fed.

## RESULTS

The studies made on natural infections of *P. reichenowi* and on No. 1 chimpanzee prior to splenectomy revealed only young rings and gametocytes in the peripheral blood, except in one natural infection where a few schizonts and trophozoites appeared when the parasitemia rose above 20,000 parasites/cu. mm. For the first 3 days of patency in No. 1, both *P. reichenowi* and *P. malariae* were visible, but *P. malariae* was not seen again during the study. Prior to splenectomy parasites were scanty and at the time of splenectomy only 2 gametocytes of *P. reichenowi* were seen. After splenectomy no parasites were visible for 14 days. On the 14th day a few rings of *P. reichenowi* appeared, and the number of rings increased slowly for 6 days. *A. gambiae* fed at this time, when 40 gametocytes/cu. mm blood were present, did not become infected. On the 6th day of patency the number of rings increased sharply and a full scale malaria attack ensued with parasites reaching and exceeding the number of half a million per cu. mm of blood in the subsequent days. The attacks lasted for 7 days when from the clinical aspect the disease died down

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although the parasitemia remained comparatively high (ca. 20,000/cu. mm blood). After a lull of 3 days a severe recrudescence took place and similar high parasitemias were recorded.

At no time were large numbers of gametocytes seen and towards the end of the recrudescence 15 gametocytes/cu. mm blood were recorded. *A. gambiae* fed at this time did not become infected. Treatment of the rec udescence with sulfonamides failed to induce any appreciable gametocytogenesis. Gametocytes persisted in low numbers for a number of days after the asexual infection had disappeared and ceased to appear only when the animal underwent prolonged sulfonamide therapy used to cure a bacillary dysentery attack.

The most striking feature of the heavy parasitemias was the appearance of the late trophozoites and schizonts in the peripheral blood. Counts made on a single generation early in the attack showed that the number of schizonts seen in the peripheral blood represented about one third of the number of rings of that generation. A strict synchronism of development allowed a reasonable estimate of the age of groups of parasites.

### *Morphology of the Parasite*

The distribution of the various characters of *P. reichenowi* at different ages as found in the splenectomised chimpanzee is shown in Tables I and II. The young parasite exactly resembles the ring of *P. falciparum*. There is little growth for the first 24 hours apart from the dispersal of the cytoplasm to form the vacuole and ring shape. During this time, however, the single nucleus of the merozoite as seen in stained smears fragments in many cases into 2 or more pieces. At the end of 24 hours as many as 50% of the rings will show 2 or more pieces of chromatin. By the time of true nuclear division at 42 hours after invasion, all of these pieces have fused to form a single whole nucleus. Appliqué forms occur as 13% of all the very young rings but are not seen after 20 hours of growth. Tenue forms are quite

TABLE I—*Percentage distribution of certain characters of* P. reichenowi

| Age of parasites in hours | Total examined | Single invasion | Double invasion | Triple invasion | Number of chromatin pieces | | | | | | Maurer's clefts | Pigment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1 | 2 | 3 | 4 | 5 | 6 | | |
| 0-3 | 500 | 80.4 | 17.6 | 2.0 | 70.6 | 11.2 | 7.2 | 5.0 | 2.6 | 3.4 | - | - |
| 15-19 | 400 | 90.5 | 8.0 | 1.5 | 52.0 | 44.5 | 3.5 | - | - | - | - | - |
| 20-24 | 200 | 84.5 | 14.0 | 1.5 | 44.5 | 45.0 | 8.5 | 1.5 | 0.5 | - | 0.5 | - |
| 36-40 | 200 | 98.0 | 2.0 | - | 64.0 | 29.0 | 7.0 | - | - | - | 42.5 | 32.0 |
| 39-41 | 100 | 99.0 | 1.0 | - | 97.0 | 3.0 | - | - | - | - | 81.5 | 99.0 |
| 43 | 200 | 99.5 | 0.5 | - | | | | | | | - | 100.0 |
| 46 | 200 | 99.5 | 0.5 | - | | | | | | | - | 100.0 |
| 48 | 100 | 99.0 | 1.0 | - | | | | | | | - | 100.0 |

TABLE II—*The number of nuclei or merozoites in schizonts of* P. reichenowi

| Age of parasite in hours | Total examined | Percentage distribution of the number of nuclei or merozoites | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| 43 | 200 | 9.0 | 16.5 | 13.5 | 23.0 | 17.0 | 16.5 | 2.0 | 2.0 | - | 0.5 | - | - | - | - | - | - |
| 46 | 200 | - | - | - | 1.0 | - | 5.5 | 1.5 | 6.5 | 5.0 | 38.5 | 6.5 | 28.0 | - | 6.0 | - | 2.5 |
| 48 | 100 | - | - | - | - | - | 1.0 | - | 8.0 | - | 26.0 | - | 47.0 | - | 13.0 | - | 5.0 |

common, usually about 10%, up to 36 hours of growth. These forms are always confined to certain portions of the thin smear.

Pigment appears in the trophozoite at about the 36th hour and shows quite suddenly as a single black mass of surprising size considering the rapidity of its appearance. The trophozoite at 36 hours is round, compact and occupies about a quarter of the red cell area. The nuclear divisions which commence at about the 42nd hour sometimes have the effect of dispersing the single mass of pigment into 2 or 3 small masses. Generally, however, the pigment remains as a single black mass throughout segmentation and does not increase much in size. When scattered into smaller aggregations it may appear brown particularly at the edges of the smallest masses.

At the late trophozoite and young schizont stage the cytoplasm stains an intense blue color although in general all stages display a more brilliant blue than most other mammalian plasmodia. The nuclei stain bright red with little or no purple overtones. The immature schizonts in rare cases show a "pseudo-cytomere" formation resulting from clefts entering the cytoplasm between the nuclei.

The mature schizont fills between one quarter and two thirds of the red cell. It measures an average of $5.6 \pm 1.0$ microns in diameter. The merozoites measure 1.2–1.9 microns in diameter. Parasites seen in whole chimpanzees conformed in every way with these descriptions.

Young growing gametocytes were seen following the first attack and these resembled in every way those seen in *P. falciparum* infections. The spindle and lozenge shapes of the young macrogametocytes were not seen. All the young forms seen were typically sausage- or crescent-shaped having a mauve cytoplasm, red nucleus and short brown bars of pigment. The mature gametocytes were typical crescents of the *P. falciparum* type.

### *Appearance of the Parasitized Red Cell*

The stained parasitized red cell is unaffected by the first 15 hours of the parasite's presence, after which time it becomes slightly decolorized to give something of the brassy color seen *in vivo* with malignant tertian parasites. Maurer's clefts begin to appear at about 26 hours and are well developed by 36 hours. At 36 hours by a combination of circumstances some of the parasitized cells resemble those infected by the 15–20-hour-old ring of *P. ovale*. Firstly at this stage all parasitized cells when stained with Giemsa are pinker than normal cells. Many contain not so much frank clefts which are a feature of the 30-hour stage as a series of faint and irregularly dispersed dots of the size, or somewhat larger than, Schüffner's dots. This appearance was noted by Schwetz (1934). It could be said that the effect is like Leishman's stippling overlaid with faint, large Schüffner's dots. Occasionally the cell is frankly mammillated. The effect seems to be caused by a mammillation not easily visible at the periphery of the cell which collects the protoplasm of the cell into small irregularly spaced nodules. These points then show as darker aggregations of the general pink color of the cell.

By 40 hours the mammillation is ofter pronounced and the concentrations of color are occasionally quite plain against the paler cell. The red cells are frequently distorted, sometimes shrunken, sometimes oval or oblong. By 44 hours the red cell is very pale and no clefts can be seen. Finally by 46 hours the red cell virtually

disappears and a blank space between normal cells is all that marks its presence except for the contained parasite.

*Clinical*

Temperature records showed a major tertian periodicity with a second minor brood of parasites causing a slight quotidian periodicity. A temperature of 103° F was recorded once in both attacks and on one occasion the temperature dropped to 97.5° F. The animal showed almost no signs of ill health and little or no changes in temperament or eating habits were noted. The white blood cell count was 32,000 WBC/cu. mm throughout the attack which though high is normal for this animal. Towards the end of the first attack many mononuclears were seen to contain pigment and later a few were seen to contain whole parasitized red cells.

## DISCUSSION

*P. reichenowi* has been established as a biologically distinct species by the cross-infection experiments of Professor J. Rodhain and his colleagues (for summaries see Rodhain, 1939 and 1941). On the other hand there exists no satisfactory morphological evidence for a specific differentiation between *P. reichenowi* and *P. falciparum*, and it cannot be said that the present study has clarified this issue.

Two points of difference have been indicated in the past:

1. The schizonts of *P. reichnowi* are seen more frequently in the peripheral blood than those of *P. falciparum*. (Peel & Rodhain 1946)
2. The merozoite numbers of *P. reichenowi* are less than those of *P. falciparum*. Rodhain & Lassman 1944, Peel & Rodhain 1946)

Although in the present study large numbers of schizonts were seen in the peripheral blood, the animal in question was splenectomized and cannot be said to have provided natural conditions for the parasite. The number of merzoites of *P. reichenowi*, 6–16, lies within the range of 6–32 usually given for *P. falciparum*. Some authorities give the merozoite numbers of *P. falciparum* as 8–15 or 16 (Castellani and Chalmers, 1919, Brumpt 1949).

Recently Hoare (1955) has discussed the taxonomic status of biological variants among the parasitic protozoa. In the absence of morphological variance he accepts certain constant biological divergences such as a strict host specificity as conferring specific status upon the variants. On this basis *P. reichenowi* can be accepted as a valid species.

This study has brought several other points into prominence. It is obvious from the figures given that most of the parasites in a cell showing muliple invasion do not live beyond the trophozoite stage, thus multiple invasion, so common among the malignant tertain parasites, is scarcely productive.

The apparent fragmentation of the nucleus of the ring stages is a curious phenomenon which should be regarded with considerable suspicion. Unfortunately wet-fixed smears were not made, for these probably would show a large single nucleus containing one or more nucleoli.[1] In dry-fixed thin smears Romanowski stains apparently color only the nucleoli leaving the remainder of the vesicular nucleus unstained. There has been some speculation in the past upon the meaning of this fragmented nucleus but the only real conclusion which can be drawn is that

[1] Studies, at present in progress, made on wet-fixed thin smears of *P. falciparum* have shown that the nucleus of the ring stage as well as the older trophozoites is whole and single.

one should never study nuclear structure in dry-fixed preparations. It is significant also that no trophozoite just prior to true nuclear division displays this fragmentation.

Another artifact in dry-fixed thin smears is certainly the tenue forms, as these are concentrated into cerain usually very thin portions of the film. No doubt strains of malignant tertian parasites will vary in their tendency to display tenue forms, but the form itself will be none the less an artifact.

The sudden appearance of a large mass of pigment in the trophozoite is obviously an illusion. In this case no doubt proper optical methods would reveal scattered fine pigment in the younger trophozoite which at a certain stage is collected fairly quickly into an easily visible mass. Certainly this is assisted by a vigorous production of pigment in the growing trophozoite but not so vigorous as to produce such a mass of pigment in the space of a few hours. This more or less sudden concentration of pigment also explains the particular brilliance of the blue stain in the cytoplasm of the late trophozoite.

SUMMARY

The complete blood cycle of *P. reichenowi* was studied in the peripheral blood of a splenectomised chimpanzee. The cycle was found to occupy 48 hours with segmentation commencing 40–44 hours after invasion of the red cells. The schizonts produced 6–16 merozoites, the typical number being 12. The significance of several forms and changes in the host cell is discussed.

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# THE EFFECT OF NICARBAZIN ON THE DEVELOPMENT OF IMMUNITY TO AVIAN COCCIDIA

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Nicarbazin has recently been described by Cuckler, Malanga, Basso and O'Neill (1955) as an anticoccidial agent for chickens. The effectiveness of nicarbazin for several species of coccidia parasitizing poultry was described by Cuckler, Malanga and Ott (1956). The objectives of the present investigation were, first, to determine the effect of nicarbazin on the development of immunity to re-infection with avian coccidia; and secondly, to ascertain the stage or stages in the coccidian life cycle which are affected by nicarbazin.

## MATERIALS AND METHODS

The chicks used in these studies were obtained from commercial hatcheries when 1 day old. New Hampshires and Tri-way white crosses of both sexes were used for experiments when 3 weeks of age. In all experiments, the groups were initially balanced for number (10 per group), weight and sex. The chicks were kept in metal, wire-floored, battery brooders in isolation quarters. The water and feed were provided *ad libitum;* an open-formula laboratory ration, described by Cuckler and Ott (1955), composed of natural feed-stuffs and supplemented with all known required vitamins, was employed. Nicarbazin (0.0125%) was thoroughly mixed into the ration just prior to use.

The strains of coccidia used were pure populations of *Eimeria tenella* and *Eimeria acervulina* isolated in our laboratory and *Eimeria necatrix,* kindly furnished by Dr. E. M. Dickinson, Oregon State College. The procedures used for preparing the homogenates and counting the oocysts were previously described by Cuckler and Malanga (1955).

In the immunity studies from 360 to 430 chicks were used in each experiment. Approximately one-half of the chickens were fed the basal ration throughout the experiment. The others were fed nicarbazin-medicated ration beginning 1 day prior to inoculation with oocysts and continuing until the 15th day of the experiment. The nicarbazin-medicated feed was then withdrawn and basal ration was fed for the balance of the experimental period. The oocyst suspensions were inoculated directly into the crop with a syringe. Either a single inoculation or 5 daily inoculations of graded numbers of oocysts were used. In the case of *E. tenella,* the primary infections were produced with either a single inoculation of 50,000 oocysts or 5 inoculations each of 2,000 or 10,000 oocysts. The infections with *E. necatrix* were produced with the same numbers as for *E. tenella,* and in addition a group received 5 inoculations of 400 oocysts. The coccidial exposures used for *E. acervulina* consisted of either a single inoculation of 500,000 oocysts or 5 inoculations each of 4000, 20,000 or 100,000 oocysts.

On the 5th and 9th days after the initial infections, 10 chicks (20 for *E. tenella*) were randomly selected and sacrificed from each of the infected control and nicarbazin-fed groups. The intestines and/or ceca were examined grossly for lesions

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characteristic of the type of coccidiosis being studied. In order to prevent variation and bias, all of the observations were conducted by one individual who was unaware of the identity of the groups under examination. The lesions were scored grossly (0 = normal, 1 = minimal, 4 = maximal) for severity. In the experiment with *E. acervulina* the small intestine, from the gizzard to the yolk stalk, was removed and homogenized with an electrically driven blender. The oocysts in the homogenate were counted by the procedure described earlier (Cuckler and Malanga, 1955). In the studies with *E. necatrix,* the ceca were removed on the 9th day after infection and were homogenized for counting the number of oocysts developing in the mucosa.

Starting on the 5th or 6th day after initial infection the feces were collected daily from the dropping pans. This was continued until the 15th or 20th day after infection. The fecal material was blended and diluted with 0.1 N sodium hydroxide solution. The suspension was mixed thoroughly with a mechanical shaker and brought to standard volume (3000 ml). Aliquots of 10 ml each were removed and mixed with an equal volume of N sodium hydroxide solution. After a period of 1 or 2 days, this suspension was "cleared" sufficiently for accurately estimating the number of oocysts produced daily by each chicken. However, with this procedure, it was possible to determine the number of oocysts present only if they were in excess of 100,000 per chicken.

On the 21st day after initial infection all surviving chickens, including those which had not previously been infected, were inoculated orally with either 100,000 oocysts of *E. tenella* or 50,000 oocysts of *E. necatrix* or 1,000,000 oocysts of *E. acervulina* of the same strain as used for the primary infection. On the 5th and 9th days after challenge to re-infection, groups of 5 or 10 chicks were sacrificed and the lesion scores were determined and oocyst counts were performed as previously. Varying slightly with the different species of coccidia, the numbers of oocysts discharged in the feces were also determined daily from the 26th to the 38th days after primary infection.

During the course of the various experiments all mortality was attributable to coccidiosis. The weight of the chickens was recorded initially and on the 21st and last days of the experiments.

The experiments designed to determine the effect of nicarbazin on the developmental stages of the avian coccidia were conducted as follows. Groups of approximately 250 chicks each were used. Equal numbers of chicks were fed basal ration or nicarbazin ration. One day after the nicarbazin ration was fed the various groups of chicks were inoculated with a single oral dose of 50,000, or 5 daily doses of 2000 or 10,000 *E. tenella* or *E. necatrix* oocysts. A similar schedule was used for *E. acervulina* but the single oral dose consisted of 500,000 oocysts and the 5 repeated doses were each composed of 20,000 and 100,000 oocysts.

Random samplings of 5 chicks each were selected from the control and nicarbazin-fed groups 2, 4, 6 or 9 days after infection with *E. acervulina.* The anterior loop of the small intestine was removed, scrapings were made from the mucosa for microscopic examination and portions were fixed in Bouin's solution. Cecal tissues were examined and fixed from similar groups of chicks 3, 5, 6 or 8 days after inoculation with *E. tenella.* In the chicks infected with *E. necatrix,* the midportion of the small intestine and the ceca were examined and portions were pre-

served in Bouin's 3, 5, 8 or 12 days after initial infection. The tissues were embedded, sectioned and stained with hematoxylin and eosin. The tissues were subsequently examined for developmental stages of the coccidia and comparisons were made between the untreated and nicarbazin-fed groups.

## EXPERIMENTAL RESULTS

### *Development of immunity*

Cecal coccidiosis caused by *Eimeria tenella.*

The effects of graded doses of cecal coccidia on mortality and cecal pathology during exposure and after immunity challenge are shown in Figure 1. These data

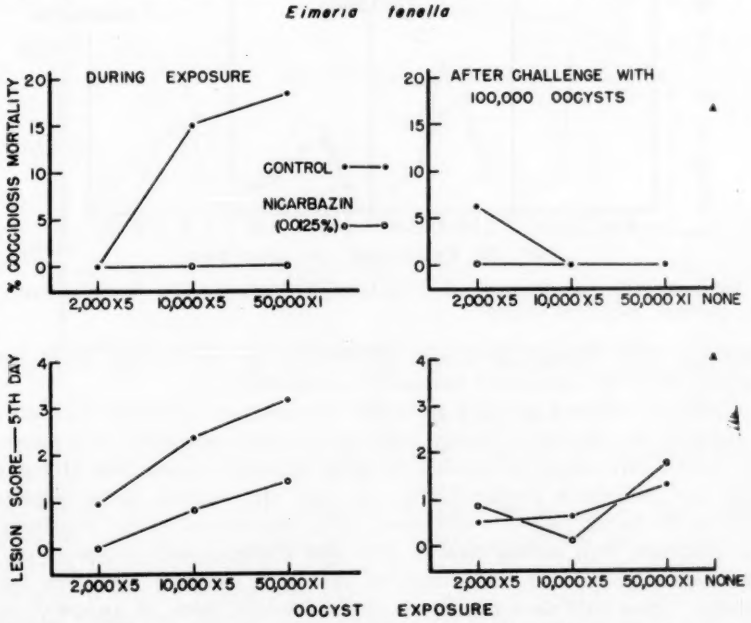



FIGURE 1. Effect of varying doses of cecal coccidia (*E. tenella*) on mortality and cecal pathology during exposure and after challenge for immunity.

show that chickens inoculated with varying numbers of oocysts and fed nicarbazin-medicated rations were completely protected from coccidiosis mortality. However, when challenged for immunity, the nicarbazin-fed chickens were equally as resistant as the infected, untreated chickens to the lethal effects of a dose of oocysts which killed 17 percent of the previously unexposed control chickens. Furthermore, the cecal pathology in the nicarbazin-fed chickens exposed to graded doses of oocysts was equal to, or lower than, that of the untreated chickens when challenged with 100,000 oocysts of *E. tenella.*

The data summarized in Figure 2 show that nicarbazin prevented or greatly reduced the bleeding which is associated with cecal coccidiosis. However, chickens fed nicarbazin and exposed to coccidiosis were equally as resistant to cecal bleeding as the unmedicated, exposed controls when subsequently challenged for

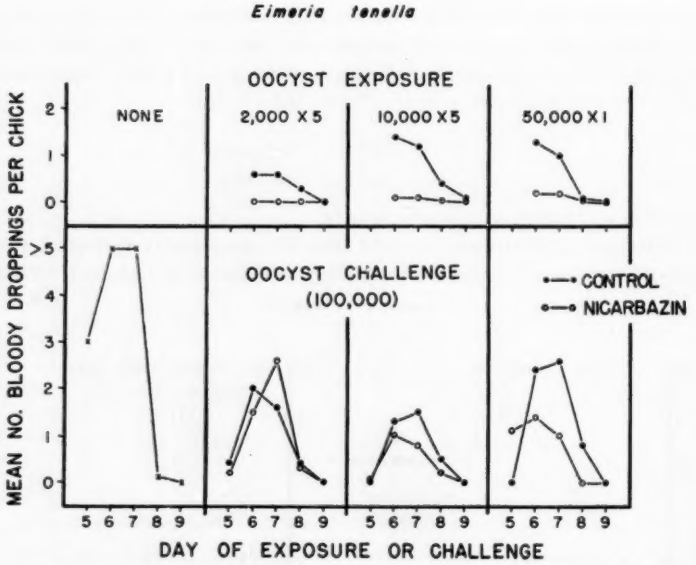



FIGURE 2. Effect of nicarbazin on the passage of bloody dropping during exposure and after challenge with cecal coccidia.

immunity. Both exposed groups of chickens passed significantly fewer bloody droppings than the unexposed susceptible control birds.

Additional evidence of the high degree of resistance to re-infection acquired by chickens fed nicarbazin during exposure to cecal coccidiosis was shown by their comparative weight gains after challenge infection. Inspection of Figure 3 shows that nicarbazin protected chickens from the marked growth-depressing effects of the largest exposure of oocysts. Furthermore, all of the nicarbazin-fed birds continued their normal rates of gain after challenge with a dose of oocysts which seriously retarded the growth of susceptible challenge infection control chickens. These data show that, on the basis of weight gains, all groups of chickens exposed to graded numbers of *E. tenella* oocysts were highly resistant to re-infection when challenged for immunity.

Intestinal coccidiosis caused by *Eimeria acervulina.*

Experiments similar to those described for *E. tenella* were also conducted with *E. acervulina.* This species of avian coccidia seldom produces mortality, but severe infections result in characteristic lesions in the anterior portion of the small intestine (Peterson, 1949). On the 5th day after infection, chickens fed nicarbazin and inoculated with 5 doses of 4,000, 20,000 or 100,000 oocysts or a single dose of 500,000 oocysts did not have detectable coccidial lesions in the intestine. The comparable unmedicated control groups had detectable to moderately severe coccidial lesions at this time. The repeated exposure to 100,000 oocysts on 5 days resulted in a lesion score of 3, whereas a single dose of 500,000 oocysts produced lesions of equivalent severity (e.g., 2.8).

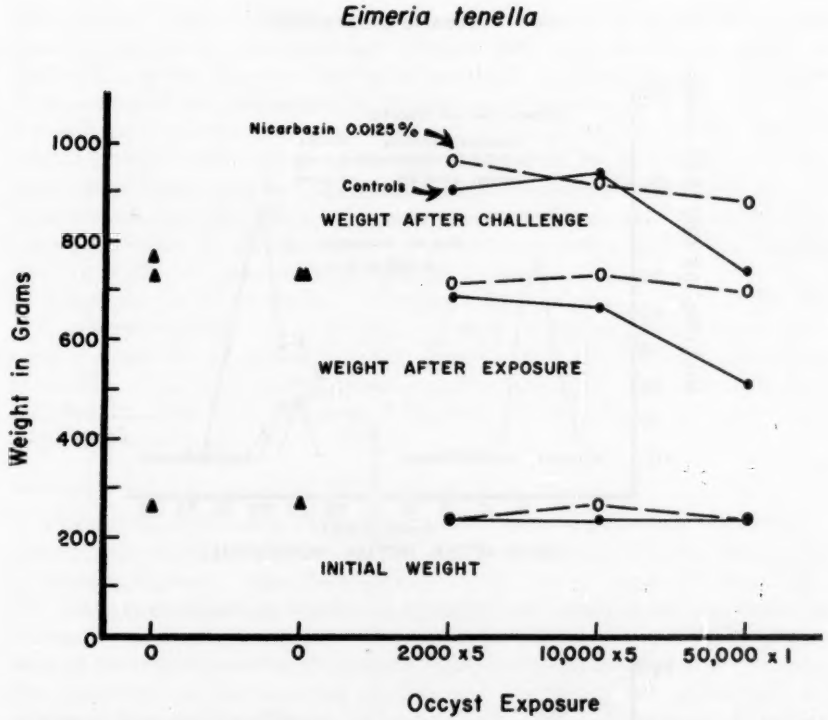



FIGURE 3. The comparative effect of graded oocyst exposures on the growth of chickens after primary and challenge infections.

All groups of birds were challenged with 1,000,000 oocysts on the 21st day after initial infection. The susceptible, previously uninfected birds showed the most severe intestinal lesions when examined on the 5th day after infection. The nicarbazin-fed and the unmedicated groups previously exposed to 4000 oocysts on 5 successive days had similar lesion scores which were considerably less than in the susceptible challenge controls. All other oocyst exposures produced maximal immunity since both the unmedicated control groups and the nicarbazin-fed groups did not have detectable lesions on the 5th day after challenge.

Although nicarbazin greatly reduced or prevented the gross lesions of coccidiosis due to *E. acervulina,* the formation and passage of oocysts in the feces was not completely prevented. However, the number of oocysts eliminated in the feces was significantly reduced. This is illustrated in Figure 4 for the repeated 4,000- and 20,000-oocyst exposure groups. The groups exposed to repeated doses of 100,000 oocysts or a single dose of 500,000 oocysts responded similarly to the lower oocyst exposure groups and, therefore, these data are not shown graphically. The smallest total number of oocysts (150 million/chick) was produced by the birds given the smallest oocyst exposure. However, the largest number of oocysts (447 million/chick) was eliminated by the birds exposed to repeated doses of 20,000 oocysts. The birds exposed to repeated doses of 100,000 oocysts produced essentially the

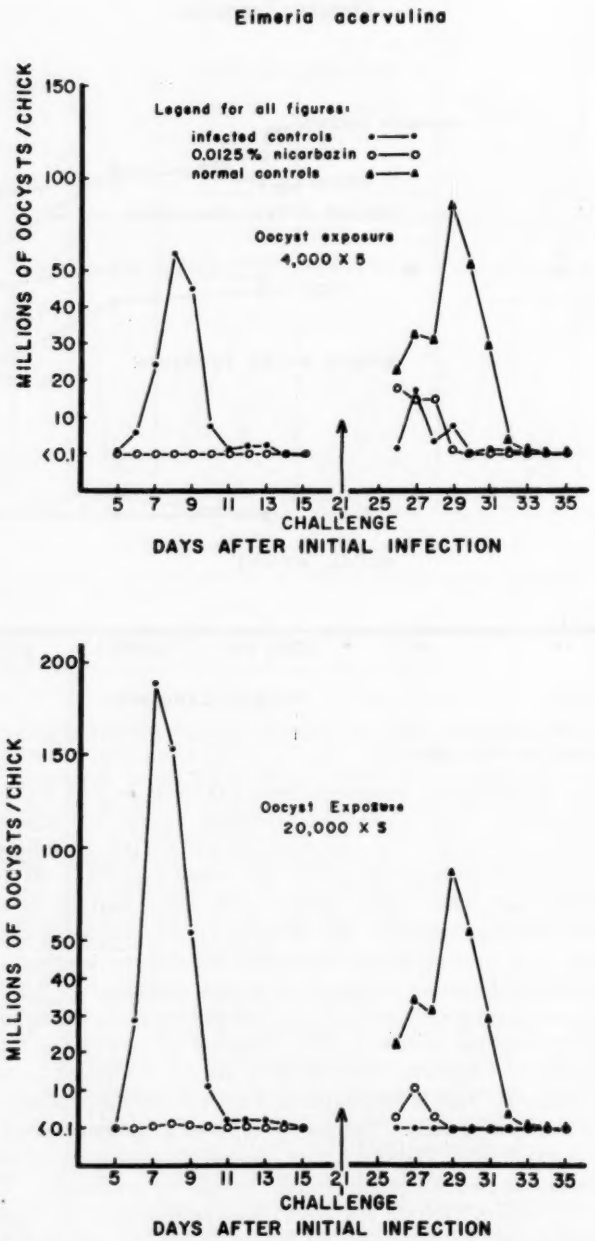



FIGURE 4. Fecal oocyst production during exposure to graded dosages of coccidia and subsequent challenge with 1 million *E. acervulina* oocysts.

same number (256 million/chick) as the single exposure with 500,000 oocysts (220 million/chick). The challenge infection with 1 million oocysts yielded a total of 260 million oocysts/chick in the previously uninfected birds. In respect to the number of oocysts produced by the birds on re-infection, all of the nicarbazin-fed birds produced about the same numbers of oocysts as the unmedicated, infected control groups. Both groups were significantly different from the susceptible challenge inoculum controls. The number of oocysts passed by the nicarbazin-fed birds was reduced to 80 to 98% less than the number in the susceptible controls. The comparable values for the unmedicated control groups varied from 87 to 99% less than the number in the susceptible challenge controls.

Under the conditions of these experiments, 5 daily doses of 20,000 or 100,000 of *E. acervulina* oocysts or a single dose of 500,000 oocysts will permit the development of a high and significant degree of immunity to re-infection with this species of coccidia. The results of the studies described have shown that nicarbazin does not adversely affect the development of immunity or resistance to re-infection with *E. acervulina* oocysts.

Intestinal coccidiosis caused by *Eimeria necatrix*.

Chickens infected with graded numbers of *E. necatrix* oocysts and fed 0.0125% concentration of nicarbazin were fully protected from the lethal effects of this species as shown in Figure 5. After challenge with a single dose of oocysts, the nicarbazin-fed chickens were equally as resistant to mortality from re-infection as the untreated exposed, surviving control birds. The data in Figure 5 also show that, on the basis of the relative severity of intestinal coccidial lesions on the 5th or 9th days after exposure, the nicarbazin-fed chickens had significantly less pathological involvement than the unmedicated, infected controls. After challenge for immunity to re-infection, the previously exposed, untreated chickens and the nicarbazin-fed birds had almost identical, low intestinal lesion scores. The lesions in both of these groups were much less severe than in the previously uninfected, susceptible controls.

Further evidence of the relatively high degree of immunity acquired by the nicarbazin-fed birds is shown in Figure 6. In respect to the number of oocysts produced and passed in the feces of exposed and challenged birds, there were no significant differences between the nicarbazin-fed and control birds, with one possible exception. In the chickens exposed to 2000 oocysts daily for 5 days and fed nicarbazin, the number of oocysts discharged was 75% less than in the susceptible controls, whereas the untreated exposed controls passed 98% fewer oocysts than the previously unexposed group. However, on the basis of the estimation of the numbers of oocysts in the cecal homogenates from these challenged chickens on the 30th and 37th days after initial infection there were absolutely no differences. Both of these groups were obviously very different in this respect from the susceptible unexposed controls (mean oocyst counts of $< 0.1$ vs $3.9 \times 10^6$).

A further indication of the protective value of nicarbazin in intestinal coccidiosis is shown in Figure 7. This diagram compares the mean body weight of the chicks in the varous groups prior to infection, after primary infection and following re-infection. Although the initial weight of all groups was essentially the same, the growth was greatly retarded in unmedicated chickens infected with repeated doses

of 10,000 oocysts or single doses of 50,000 oocysts. However, all nicarbazin-fed chickens gained equally as much as the unexposed control birds. Likewise, the untreated control chickens which were exposed to repeated infections with 400 or 2000 oocysts made good weight gains.

When all birds, including the unexposed normal birds, were challenged on the 21st day, the birds previously fed nicarbazin did not suffer the decreased rate of gain shown by the susceptible control birds. In fact, all groups of nicarbazin-fed

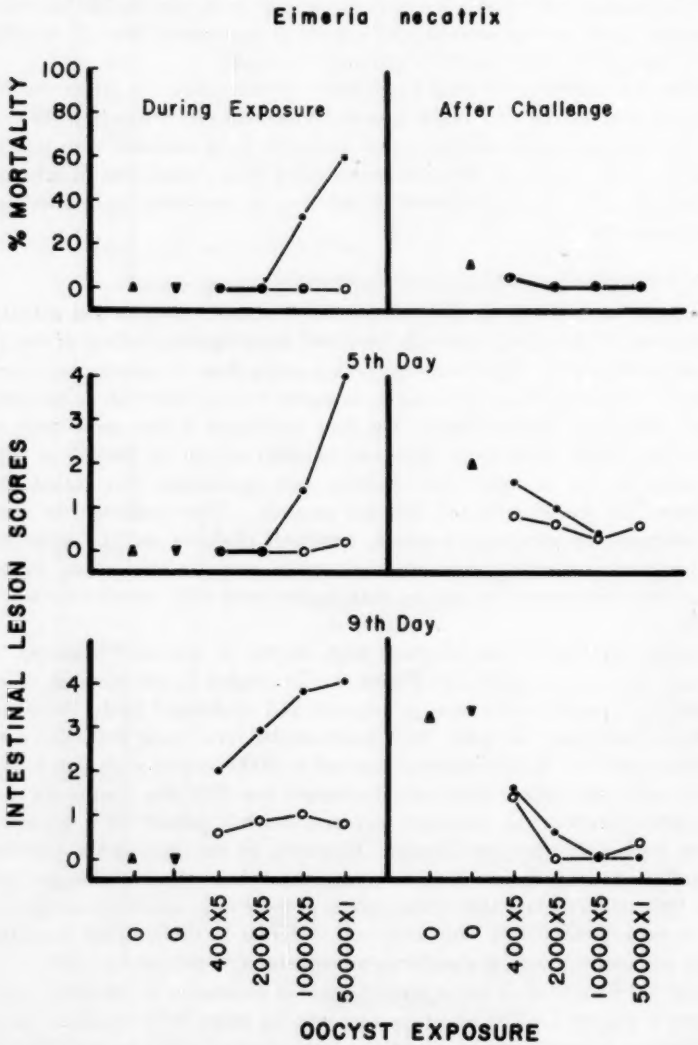
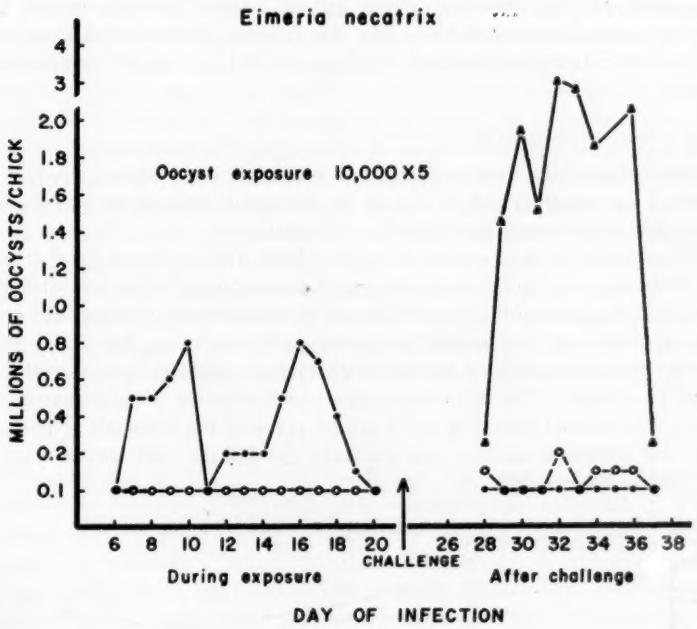



FIGURE 5. Effect of graded doses of *Eimeria necatrix* oocysts on mortality and intestinal pathology during primary infection and after challenge for immunity. (Legend same as in Fig. 4)



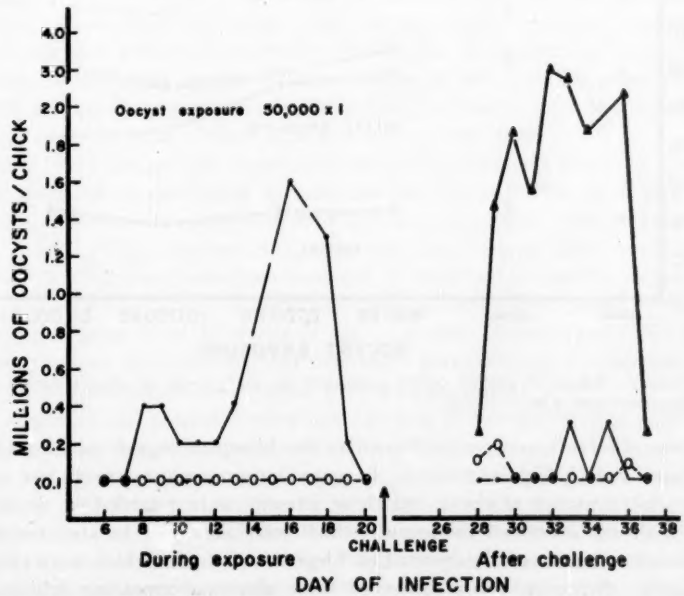




FIGURE 6. Fecal oocyst production during exposure to graded dosages of coccidia and after subsequent challenge with 50,0000 *E. necatrix* oocysts. (Legend same as in Fig. 4)

birds maintained their excellent rates of growth. Since there was neither retardation in growth-rate nor loss in weight this is interpreted as additional evidence that nicarbazin permitted the full development of immunity to re-infection with coccidia.

Site of action of nicarbazin

Examination of the fresh scrapings of intestinal or cecal mucosa and histological sections of the intestines and/or ceca of the infected nicarbazin-fed and the infected control chickens revealed the following information.

The intestinal or cecal mucosa from the chicks fed nicarbazin gave evidence of some thickening and rarely some very small hemorrhages. This indicated that the tissues had been invaded by the coccidia and an inflammatory reaction had occurred. However, relatively few second generation schizonts were found in the fresh scrapings from the mucosa of the intestinal tracts of chickens infected with coccidia and fed nicarbazin. The schizonts present were retarded in development. After a longer than normal period of development, some of the schizonts contained fully formed and motile merozoites. Occasionally gametocytes and oocysts were found in the nicarbazin-fed chickens.

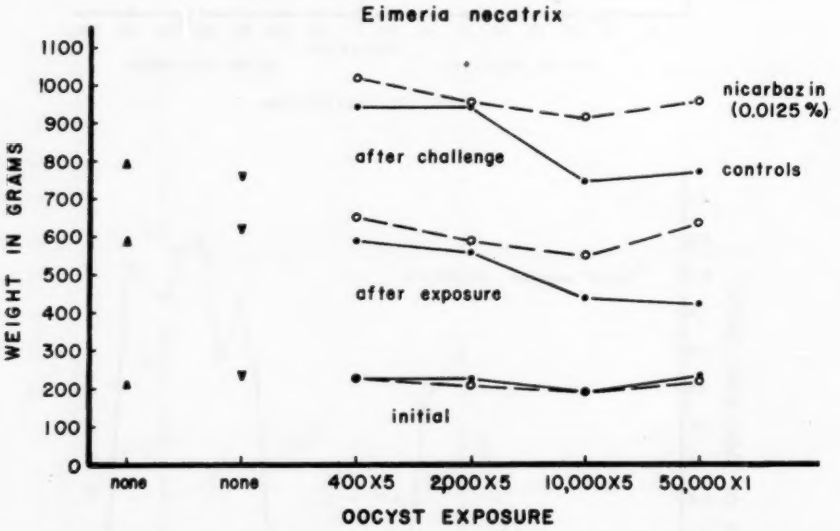



FIGURE 7. Effect of graded oocyst exposures on the growth of chicks during exposure and after challenge with coccidia.

These observations were confirmed in the histopathological sections as shown in Plates I and II, Figures 8 to 17. In general, there were relatively few schizonts in the nicarbazin-fed chickens and those present were retarded in development. Some schizonts contained merozoites which were abnormal in size, number and appearance. These were interpreted as "degenerate" forms which were affected by nicarbazin. However, the presence of these abnormal-appearing schizonts produced a greater inflammatory reaction in the tissues than observed in the

infected control chicks. Furthermore, the extensive sloughing of the mucosa and the profuse bleeding which accompanies cecal coccidiosis did not occur in the nicarbazin-fed chickens. In fact, it appears that the tissues retain the parasites after they are killed by nicarbazin and then a relatively severe foreign-body reaction occurs. This culminates in some instances with giant-cell formation.

From the examination of these tissues, it is concluded that nicarbazin has its greatest effect on the second generation schizonts and their merozoites. However, the presence of these and earlier stages in the coccidian cycle permitted the development of a relatively high degree of acquired immunity to re-infection in chickens fed nicarbazin. Indeed, the lethal effect of nicarbazin on many of the second generation schizonts may be responsible for stimulating the high immune response. It would appear, therefore, that the feeding of nicarbazin to chickens permits the development of a high degree of immunity to re-infection with coccidia without the severe pathological effects and sequelae which accompany coccidiosis in unmedicated birds.

## DISCUSSION

The development of immunity to re-infection with coccidia was first clearly demonstrated by Johnson (1927) and later confirmed and extended by Tyzzer, *et al.* (1932) and Dickinson (1941). Since these classical studies, numerous other investigators have added to our information on immunity in coccidial infections. Until the introduction of the sulfonamides and the subsequently discovered anti-coccidial agents, the poultry raiser was largely dependent upon management practices and acquired immunity to protect the flock from the severe outbreaks and high mortality of coccidiosis. The studies by Delaplane *et al.* (1947) with sulfaquinoxaline demonstrated that the mortality and pathological sequelae of coccidiosis could be controlled without significantly affecting the development of immunity to re-infection. Subsequent studies by others have shown that the more recently discovered anticoccidial agents would also permit the development of immunity to re-infection with coccidia. Nicarbazin also follows this pattern.

Barber (1955) has recently shown that nicarbazin effectively protected chickens from the mortality of coccidiosis and did not interfere with the development of immunity to re-infection with *E. tenella* and *E. acervulina*. Similarly, White-Stevens *et al.* (1955) reported that nicarbazin and several other coccidiostats allowed the development of adequate immunity to cecal and intestinal coccidiosis in pen-reared chickens as shown by protection from mortality after massive challenge when the birds were 10 or 12 weeks of age. Cuckler, Malanga and Ott (1956) have also presented data showing that nicarbazin permitted the development of a high degree of immunity to re-infection with cecal coccidia.

The results of the present studies have further demonstrated that nicarbazin does not interfere with the acquisition of coccidial immunity. The data presented above are largely self-explanatory and there is little need for additional comment. However, the importance and relationship of the number of oocysts and the number of infections in stimulating a high degree of immunity to re-infection with coccidia cannot be over-emphasized. Our studies have confirmed that the relative degree of immunity developing to re-infection with coccidia is directly dependent upon these two factors. Under experimental conditions, these variables can be controlled.

However, under practical conditions of poultry management, there is no control over these factors. It is generally assumed that, under practical conditions, chickens are exposed to repeated infections with relatively small numbers of oocysts. Although very light infections may fail to confer demonstrable resistance to re-infection with coccidia, single or repeated infections with a given number of oocysts will permit chickens to survive later exposure with infections which are lethal for fully susceptible birds.

The data presented above have shown that chickens fed nicarbazin developed the same relative degree of immunity as the unmedicated control chickens given similar exposures of coccidia. The way in which nicarbazin affected the coccidian life cycle and the response this provoked in the tissues of the host appears to explain the high degree of immunity found.

## SUMMARY

Chickens exposed to infections with graded numbers of *E. tenella*, *E. acervulina* or *E. necatrix* oocysts and fed nicarbazin were equally as resistant as infected, untreated surviving control chickens to the lethal effects of a challenge infection.

The feeding of nicarbazin significantly reduced the damaging pathological effects of avian coccidiosis but did not interfere with the development of acquired immunity.

Although nicarbazin significantly suppressed oocyst formation, this did not adversely affect the immunizing process produced by avian coccidia.

Histopathological studies have shown that nicarbazin suppressed the development of the second generation schizonts and their merozoites. The presence of these retarded schizonts produced a marked inflammatory reaction in the intestinal tissues. This reaction appears to have stimulated immunity and may account for the relatively high degree of resistance to re-infection with coccidia found in chickens fed nicarbazin.

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EXPLANATION OF PLATES I AND II

Effect of nicarbazin on the development of coccidia. All tissues stained with hematoxylin and eosin. Magnification 280×. *Eimeria tenella:* FIG. 8. Unmedicated control, 7th day after infection with 50,000 oocysts. Numerous second generation schizonts in various stages of maturity. Note also the developing trophozoites and gametocytes in adjacent cells.

FIG. 9. Similar cecal tissues from nicarbazin-fed bird on 7th day after infection with 50,000 oocysts. The appearance of second generation schizonts. Note particularly the small number of schizonts and the inflammatory reaction to their presence.

*Eimeria necatrix:* FIG. 10. Mid-portion of small intestine from unmedicated control chicken on the 7th day after infection with 50,000 oocysts. Note the mature second generation schizonts in submucosa.

FIG. 11. Similar tissues from nicarbazin-fed chicken on 7th day after infection with 50,000 oocysts. Note that the schizonts are less-well developed and there is an inflammatory reaction in the tissues.

FIG. 12. Developing trophozoites and gametocytes in cecal tissue of control chicken on the 7th day after infection with 50,000 oocysts.

FIG. 13. Comparable cecal tissue from nicarbazin-fed chicken.

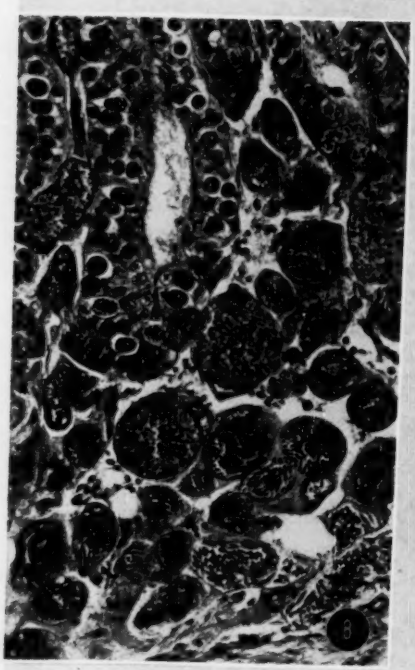
*Eimeria acervulina:* FIG. 14. Portion of small intestine from unmedicated control chicken on 4th day after infection with 500,000 oocysts. Note numerous schizonts in superficial epithelium.

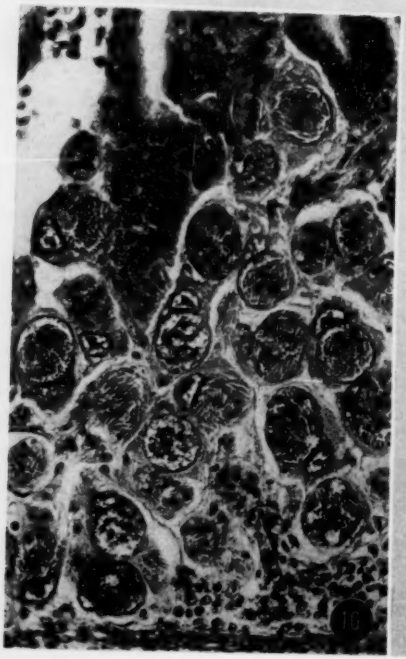
FIG. 15. Intestinal tissues from nicarbazin-fed chicken on 4th day after infection with 500,000 oocysts. Schizonts are present in epithelium.

FIG. 16. Intestine from control chicken on 6th day after infection with 500,000 oocysts. Numerous developing trophozoites, gametocytes and oocysts in mucosa.

FIG. 17. Similar tissue from nicarbazin-fed chicken. Note presence of exudate and inflammatory reaction.

# Plate I





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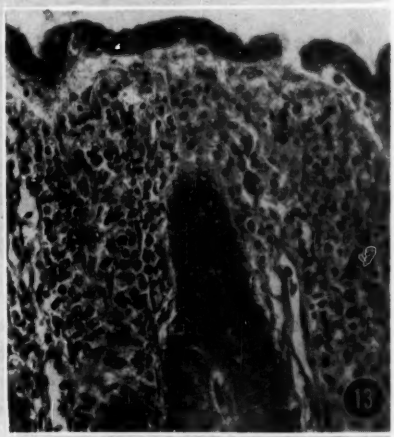

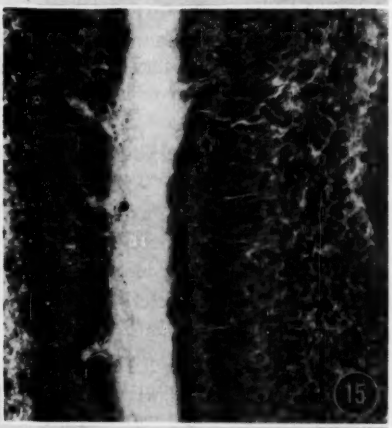


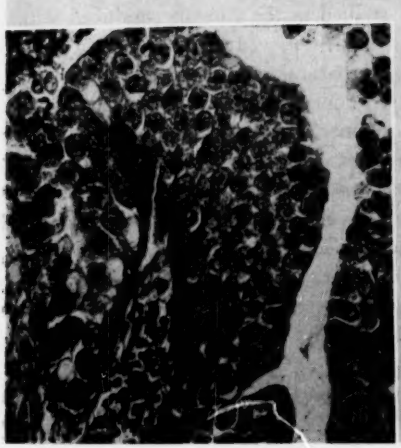

# EFFECT OF SINGLE INOCULA, OF VARIED SIZE, ON THE RESISTANCE OF HAMSTERS TO *TRICHINELLA SPIRALIS*[1]

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Little information is available on the host-parasite relationships of hamsters to *Trichinella spiralis*. Boyd and Huston (1954) attempted to ascertain the susceptibility of the hamster to *Trichinella* by inoculating 2 groups of animals with 100 and 500 larvae, respectively. Their results indicated that the ratio of developing larvae to dosage was much greater when the larger inoculating dose was used. These findings are in apparent contradiction to those of Marchant (1939) who found that, in rats, the percentage recovery of larvae became progressively less as the dosage increased.

In order to study further these and other aspects of the resistance of hamsters to infection, groups of animals of the same age were compared, each animal having received a single inoculation of from 125 to 8,000 viable larvae.

## MATERIALS AND METHODS

Eleven-week-old female hamsters of approximately the same weight were used for these experiments. White rats were used as viability controls of the *Trichinella* larvae; they were sacrificed 30 days after inoculation and examined for the presence of larvae in the muscles. All rats became infected as a result of the inoculation. All animals were inoculated by stomach tube with larvae isolated from trichinous rat muscle as described in a previous paper (Sadun and Norman, 1955). The experimental animals were kept in wire-screened cages, fed rat pellets and cabbage, given water *ad libitum*, and weighed every week. Necropsy was performed on most of the dead animals in an attempt to determine whether death might have been produced by causes other than trichinosis.

The intestinal phase of parasitism was studied in the animals sacrificed within 8 days after inoculation. The entire intestine was removed at necropsy, slit, placed over a wire screen in a modified Baermann apparatus containing saline at 37° C, and kept in an incubator for approximately 5 hours. The *Trichinella* adults were collected at the tip of the funnel and their number was ascertained by direct or by dilution count. The larvae in the muscles were recovered by mincing the meat and bone of the hamsters in a Waring Blendor for 1 minute and incubating for approximately 20 hours in artificial digestive juice at 37° C. The larvae were collected at the tip of the funnel and their number was ascertained by dilution counts.

Details of dosages will be given in the descriptions or tables of the experiments reported below.

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## RESULTS AND DISCUSSION

### 1. *Lethal Infections*

The results of the effect of graded infections on the survival of hamsters are summarized in Table I. The relation between infection and weight was already

TABLE I— *Effect of graded inocula of* T. spiralis *on the survival of hamsters*

| Group Number | No. of Animals | No. of Larvae Per Animal | Per Cent Mortality* | Average Time of Survival |
|---|---|---|---|---|
| I | 10 | 0 | 2 | — |
| II | 10 | 125 | 3 | — |
| III | 10 | 250 | 15 | 43 |
| IV | 10 | 500 | 24 | 32 |
| V | 10 | 1,000 | 61 | 31 |
| VI | 10 | 2,000 | 86 | 31 |
| VII | 10 | 3,000 | 97 | 28 |
| VIII | 10 | 4,000 | 100 | 15 |
| IX | 10 | 6,000 | 100 | 14 |
| X | 20 | 8,000 | 100 | 11 |

*Estimated by accumulation of deaths and survivals (Reed and Muench, 1938)

noticeable 1 week after inoculation and became more striking as the experiment proceeded. Most of the hamsters that had received between 4,000 and 8,000 larvae (Groups VIII, IX, and X) began losing weight during the first week following inoculation. By the third week, the mean weight in all the experimental groups had begun to decrease, whereas that of the uninfected controls (Group I) continued to increase. Altogether, 67 hamsters died apparently as a result of the infection. No obvious cause of death could be determined in 1 control animal which died after 19 days. Death in the experimental animals occurred between the third and the 45th day after inoculation, with an average of 20.3 days. The greatest number of hamsters, however, died during the second week (17) and the fifth week (14). All of the animals that died during the second week were in the groups inoculated with very heavy doses (between 4,000 and 8,000 larvae each); whereas, all of those that died during the fifth week came from the groups which received somewhat milder doses (between 500 and 3,000 larvae, each). This frequency distribution in time from inoculation to death shows a correlation between the time of death and the life cycle of the parasite, namely, the intestinal and the muscular phases. This observation is strengthened by the fact that animals receiving 4,000 larvae or more began losing weight almost immediately whereas those receiving between 500 and 3,000 larvae gained weight during the intestinal phase and began losing it during the muscular phase. The $LD_{50}$ was estimated by the method of Reed and Meunch to be 850 larvae. In general, the size of the inoculum was found to have an inverse relation to the average time between inoculation and death. While not always significant from one size of inoculum to another, the difference in average survival time of the hamsters that received 250 larvae (Group III) and of those that received 8,000 larvae (Group X) is highly significant.

In groups III to VII the average weight at the time of inoculation of those hamsters which later succumbed to the infection was not appreciably different from that of the animals which survived.

2. *Relation between Size of Inoculum and Infection*

Hamsters that had received nonlethal, single infections were sacrificed 30 days after inoculation and the carcasses were digested. The results are summarized in Table II.

**TABLE II — *Effect of graded inocula of* T. spiralis *on the development of larvae***

| Number of Animals | Size of Inoculum | Larvae in Muscles at Necropsy 30 Days After Inoculation | | | |
|---|---|---|---|---|---|
| | | Minimum No. Per Animal | Maximum No. Per Animal | Average No. Per Animal | Ratio to Dosage |
| 26 | 125 | 5,700 | 97,900 | 32,250 | 1:258 |
| 27 | 250 | 3,250 | 150,000 | 78,190 | 1:313 |
| 26 | 500 | 29,700 | 272,250 | 161,180 | 1:322 |
| 27 | 1,000 | 70,400 | 382,000 | 245,060 | 1:245 |

Increase in size of inoculum brought about an increase in the mean number of larvae recovered. However, whereas in the first 3 groups the ratio to dosage showed an apparent increase with the size of inoculum, in the group receiving 1,000 larvae there was a decrease. Table II also shows that considerable variations existed in the numbers of larvae recovered among animals within the same group. An analysis of variance of the ratio between the average number of larvae recovered in each group and the size of the inoculum showed no significant differences between different sizes of inoculum (F for different inocula being equal to 2.84, whereas an F of 8.56 is required for significance at the 5% level). These results are in agreement with those reported by McCoy (1931) in rats, and Boyd and Huston (1954) in hamsters. No quantitative comparison can be made of the data presented above with those in the literature because of the differences in the age of the experimental animals, in the size of the infective doses, and in the duration of the infection. However, some general comparisons may be justifiable. The numbers of larvae recovered in the above experiments from the hamsters inoculated with 125 and 500 larvae are much greater than those reported by Boyd and Huston (1954). This difference may be ascribed to the difference in the age of the host at the time of inoculation, since they used hamsters which were about 17 weeks (120 days) old. The hamsters in our experiments were only 11 weeks old at the beginning of the experiment. This suggests the presence of an age resistance to *T. spiralis* in hamsters which operates somewhat similarly to that which has been reported for other hosts.

3. *The Effect of Graded Inocula on the Development of Adults*

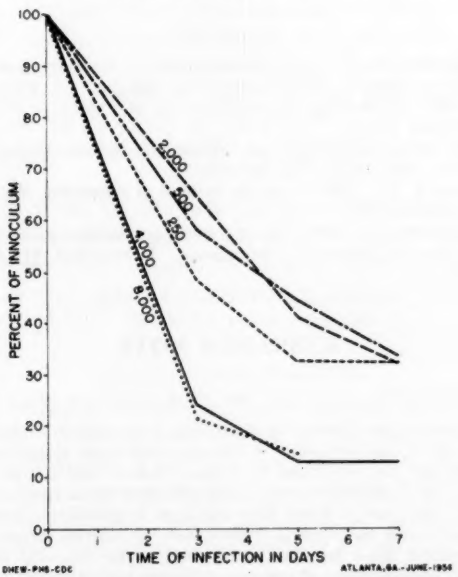
The results are summarized in Table III and presented graphically in Figure 1. In all groups, there was a gradual decrease in the percentage of recovery of adults as the time from inoculation increased. Three days following inoculation about one half as many adults as the number of larvae inoculated could be recovered in the groups receiving a dose of 2,000 larvae or less. On the other hand, by the same time less than one fourth as many adults as the size of the inoculum could be recovered in the groups receiving 4,000 larvae or more. Three days following inoculation, the highest recovery was observed in hamsters receiving 2,000 larvae each (64.5%); 5 and 7 days following inoculation, in those receiving 500 larvae

TABLE III — *Effect of graded inocula of* T. spiralis *on the percentage recovery of adults*

| Number of Animals | No. of Larvae Inoculated | Mean Number Adults Recovered | | | Per Cent Recovered | | |
|---|---|---|---|---|---|---|---|
| | | 3 Days | 5 Days | 7 Days | 3 Days | 5 Days | 7 Days |
| 28 | 250 | 121.0 | 81.5 | 81.1 | 48.4 | 32.6 | 32.4 |
| 24 | 500 | 291.3 | 223.2 | 169.2 | 58.3 | 44.6 | 33.8 |
| 24 | 2,000 | 1,291.4 | 821.9 | 645.1 | 64.5 | 41.1 | 32.2 |
| 20 | 4,000 | 963.5 | 521.2 | 525.8* | 24.1 | 13.0 | 13.1* |
| 10 | 8,000 | 1,701.0 | 1,176.0** | — | 21.3 | 14.7** | — |

*Only four hamsters were included in this group.
**Only two hamsters were included in this group.

Figure 1
THE PERCENT RECOVERY OF *T. spiralis* ADULTS IN RELATION TO SIZE OF INOCULUM & LENGTH OF INFECTION




each (44.6% and 33.8%, respectively). Attention is called to the fact that the percentage of recovery of adults in relation to the size of inoculum increased up to a certain threshold and decreased in animals receiving very heavy inocula. In the latter groups, the greatest loss of worms occurred during the first 3 days. These results are consistent with and support those obtained in connection with the effect of size of inoculum on the weight loss, on the time of death and on the relative numbers of larvae developing in the muscles. These results suggest that with very high inocula, there is a considerable sloughing of the intestinal mucosa with consequent elimination of adults, reduction in the production of larvae in the muscles, loss of weight of the animals and, frequently, death during the intestinal phase of the infection.

SUMMARY

Results of several experiments involving the use of 362 hamsters of approximately the same age and weight inoculated with graded single inocula up to 8,000 larvae indicate that hamsters are susceptible to infections with *Trichinella spiralis*. No significant differences in the ratio between the average number of larvae recovered and the size of inoculum was observed in groups receiving between 125 and 1,000 larvae. The time of the greatest worm elimination from the intestinal tract varied with the size of inoculum and occurred during the first week following inoculation.

The $LD_{50}$ of hamsters of the age and size used was about 850 larvae. The size of the inoculum had an inverse relation to the average time of survival. In general, death occurred primarily at the time of the muscular phase when the inoculum was 3,000 larvae or less, and at the time of the intestinal phase when the inoculum was 4,000 larvae or more. The regularity of the results suggests the possibility of using lethal infections as an additional tool for immunological and chemotherapeutic studies.

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## RESEARCH NOTE

### EXOERYTHROCYTIC STAGES OF *PLASMODIUM NUCLEOPHILUM*

Through the kindness of Dr. Fruma Wolfson-Max I was able to study sections of the spleen of a canary infected with *P. nucleophilum*. A few exoerythrocytic stages were observed. These were sufficiently like the ones described by Muniz, Soares and Batista (1951, Rev. Brasil Malariol. **3**: 339–356) for *P. huffi* as to cast significant light upon questions raised in my study of the latter parasites (1953, Am. J. Trop. Med. and Hyg. **2**: 620–623). Although only 3 sections were available and the fixation and staining of these were not entirely satisfactory the exoerythrocytic stages seen resembled those described for *P. huffi* in the following particulars: (a) They were predominantly in lymphocytes; (b) mature schizonts were spherical in shape and brought about enlargement of the host cell, and (c) the number of merozoites exceeded the number normally found in *P. elongatum* (8–12). In my comments on *P. huffi* (1953) I called attention to Dr. R. D. Manwell's opinion that the erythrocytic stages seen in the preparations of *P. huffi* made by Muniz, Soares, and Batista were sufficiently like those of *P. nucleophilum* to suggest the possibility that the Brazilian workers might have been dealing with a mixed infection. The observations here recorded on the exoerythrocytic stages of *P. nucleophilum* indicate the more likely possibility that *P. huffi* may be closely related to or identical with *P. nucleophilum* since both erythrocytic and exoerythrocytic stages are similar in the 2 species. A more careful comparison is needed to settle this point. Unfortunately no strain of either parasite is at present available for study.—CLAY G. HUFF, *Naval Medical Research Institute, Bethesda, Md.*

# STUDIES ON EGYPTIAN TREMATODES WITH SPECIAL REFERENCE TO THE HETEROPHYIDS OF MAMMALS. II. EMBRYONIC DEVELOPMENT OF *HETEROPHYES AEQUALIS* LOOSS[1]

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Japanese investigators were the first to describe the life cycles of heterophyid trematodes, e. g., *Metagonimus yokogawai* by Yokogawa (1913) and *Stamnosoma formosanus* by Nishigori (1924). Two more cycles, those of *Monorchotrema taihokui* and *M. taichui* (now *Haplorchis pumilio* and *H. taichui*) were described by Faust and Nishigori (1926). In 1930, Stunkard described the life cycle of *Cryptocotyle lingua* and Cable added further details in 1934. Witenberg (1929) obtained a number of heterophyids from laboratory feedings in Palestine, and Khalil (1932, 1937) gave a general account of the life histories of *Qasr ainy* and *Heterophyes heterophyes,* respectively, in Egypt. Ameel (1938) described the life cycle of *Euryhelmis monorchis* to which he, Cort, and Van der Woude added details in 1950. Only in the works of Cable (1934) and Ameel, Cort, and Van der Woude (1950) have details of embryonic development in the molluscan hosts been described.

In Part I of this series (Kuntz and Chandler, 1956) the taxonomic relationships of some of the more common heterophyids of the Middle East were discussed, including those of *Heterophyes aequalis,* of which *H. dispar* is believed to be a synonym. The present paper gives a description of the embryonic development of *H. aequalis* in the snail host, *Pirenella conica* Blainville.

## MATERIALS AND METHODS

Developmental stages of *H. aequalis* were obtained from naturally infected *Pirenella conica* taken from the mud and silt in the shallow, brackish water of Lake Burullus. The majority of molluscs was taken at the edge of the village of El Burg, near the channel connecting the lake with the Mediterranean. Water in this region has a greater salt content than water in more distant parts of the lake. Naturally infected cats were also collected at El Burg.

Identification of flukes was based upon a study of adults obtained from laboratory-reared kittens which were fed metacercariae teased from the musculature of laboratory-raised *Gambusia affinis* infected with selected heterophyid cercariae. *Pirenella* with cercariae of the type herein described as *H. aequalis* were separated from snails from which other types of cercariae were emerging. Cercariae were pipetted into crystallizing dishes or finger bowls containing ½ inch of filtered, brackish water from Lake Burullus. One *Gambusia* was introduced into each dish. *Gambusia,* normally of fresh water, were previously conditioned to brack-

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[1] The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

ish water by gradually increasing salinity over a period of 4 to 6 hours and after a 1-hour exposure were reconditioned to aerated fresh water. The cercariae were observed to penetrate actively into the body of the fish during the period of exposure. *Gambusia* were retained 4 to 6 days before metacercariae were removed from the musculature and fed by mouth to kittens. Adult worms were recovered from the small intestine of kittens autopsied 12 to 20 days later.

*Pirenella* were not reared in the laboratory but naturally infected snails remained alive for 3 to 5 months in water from Lake Burullus. Snails seemed to obtain some food from the stratum of lake mud in each aquarium. Some snails fed upon boiled, decaying lettuce or upon small samples of formed human feces placed in the water. Maximum aeration of water was essential.

Developmental stages of rediae and cercariae were studied in the living state. Crushed snails provided excellent study material for several days when placed in a refrigerator. Certain aspects of development were checked in haematoxylin-stained sections.

## EMBRYONIC DEVELOPMENT

*Rediae.* Numerous rediae in different stages of development were present in each snail. The majority were daughter rediae producing cercariae. A few, however, 6 in several hundred observed, were large, sacculate forms producing daughter rediae and cercariae simultaneously. Although such a relationship has been reported only infrequently, the fact that we failed to find mature rediae producing only daughter rediae, indicates that these large double-producers are probably true mother rediae. It is difficult to ascertain whether all mother rediae of this species produce both daughter rediae and cercariae.

Figures 1 and 13 (Plates I and II, respectively) show that in these double-producing mother rediae there are 2 separate sites of embryo-production. As far as could be determined, cercariae developed only in the usual manner from germinal cells in the posterior part of the redia. Daughter rediae, on the other hand, apparently developed only from the portions of the lateral wall of the body cavity. In numerous daughter rediae in the same snails, where only cercariae were present, cercarial embryos were located only at the posterior end of the body cavity.

A study of the mother rediae revealed the functional relationship of birth pore, birth pouch and the body cavity, and the passage of cercariae to the exterior. In lateral view and under slight coverglass pressure, the birth pore appears as a well developed epithelium-lined chamber communicating internally with the body cavity. The birth pore at the outer end of the pouch is provided with prominent cuticularized lips. The pouch functions as a uterus and is surrounded by muscular tissues. Entry of a cercaria (presumably true also for daughter redia) into the inner end of the pouch, head or tail first, seems to initiate a series of muscular contractions which, accompanied by movements of the cercariae, hasten birth. During the delivery process, the outlines of muscles (Fig. 14) becomes more apparent and some could be traced to their points of origin some distance from the birth pouch. Secondary muscles associated with the anterior walls of the body also contract and facilitate movement of a cercaria into the inner opening of the birth pore.

The redial embryos located in the lateral body walls of mother rediae (Fig. 1) stand out as growing masses on broad-based peduncles. This effect is lost, however, as the mother redia, under coverglass pressure, is compressed due to desiccation. There is a single or several daughter rediae in each peduncular mass (Figs. 1 and 2). In the larger masses, there are several developmental stages as well as moderately well defined germinal masses, presumably destined for growth into more redial embryos. The mass in which the daughter rediae have their origin contains rather prominent vacuoles and deposits of pigment which are not apparent posteriorly at the site of cercarial production. Walls of the mature daughter rediae, as well as those in mother rediae, are loosely constructed and possess large vacuoles between the body wall proper and the lining of the body cavity. This vacuolated condition is unusually pronounced.

The vacuoles are smaller and less numerous in the walls of the young and middle-aged rediae. Nuclei are present in the tissues around the periphery of the vacuoles or fluid-filled spaces. Similar but smaller vacuoles account for the spongy nature of the parenchyma in the posterior end of rediae where early cercarial embryos are located, whether in mother or daughter rediae. Large vacuoles in the parenchyma housing the redial embryos appear similar to vacuoles in the body wall. Redial embryos are enveloped in a vitelline membrane (Fig. 2) and the peduncular mass is covered by a nucleated membrane which is a continuation of the lining of the body cavity.

In the young daughter redia differentiation of cells which form the pharynx and intestine is followed by the appearance of the next generation of germinal cells at the posterior end of the body. They differ only slightly in appearance from the surrounding body cells. The intestine at this stage has thick walls with prominent nuclei and occupies a large part of the embryo. There are accumulations of semi-refractile material in the fluid contents of the intestine and a mass of gelatinous consistency at the junction of pharynx and esophagus. Coarsely granular glands are situated at the side of the intestine. Three pairs of glands with nuclei and ducts leading to the base of the pharynx were observed in several specimens. These glands are similar in appearance to the penetration glands of the mature cercaria. As the daughter redia enlarges the pharynx becomes moderately well developed, the intestine comparatively smaller and only remnants of gland cells remain. The lining of the pharynx is finely spined and cuticularized to near the junction with the intestine (Fig. 4). Posteriorly a body cavity and numerous germinal cells and/or masses appear.

In actively producing daughter rediae, the body cavity is packed with 20 to 35 cercarial embryos in different stages of development (Fig. 5). Yellow-brown to golden pigment becomes more apparent as the rediae enlarge, and rather prominent vacuoles develop between the walls of the redia and the body cavity. Daughter rediae at the peak of cercarial production contain up to 60 developing cercariae free in the body cavity.

The excretory system is difficult to follow in the developing daughter rediae. On one occasion active flame cells were observed in the body of a young daughter redia while still attached to the wall of the mother redia. The greater part of the excretory system could be traced in one of the mother rediae (Fig. 13). In this case, only 4 pairs of flame cells could be seen, all in the anterior half of the redia.

There were 2 large dilatations in the collecting ducts. These specimens measured approximately 2.7 mm in length and moved more sluggishly than mature daughter rediae.

*Cercariae.* In living as well as in sectioned rediae, the germinal cells which give rise to cercariae appear singly or in groups in the posterior parenchyma of the body. Cells which have divided and begun embryonic development are located in the well defined spaces of less compact tissues nearer to the body cavity and differ slightly in appearance from the surrounding body cells. A vitelline membrane or embryonic envelope is present at the 8- and 16-cell stages and apparently is derived from an earlier cleavage of the embryonic cells. As the embryos grow, they move in the loose parenchyma and eventually bulge anteriorly under the lining of the body cavity. Older embryos protruding well into the body cavity are covered temporarily with 2 membranes. The inner, vitelline membrane, remains around the embryo, whereas the outer nucleated membrane which is merely an extension of the body cavity lining, remains behind as the cercariae break free. The extent to which cercarial embryos form peduncles seems to vary with age and productive activity, and is more apparent in the older mother rediae with limited numbers of cercariae. Some cercariae become free in the body cavity in early stages (Fig. 7) while others remain attached until fundaments of the oral sucker, acetabulum and other organs have appeared.

There is considerable differentiation of organs in the cercariae before the excretory system has progressed beyond the 4-flame-cell stage (Figs. 8 and 9). Cells of the pharynx appear and have begun differentiation and 2 pairs of flame cells are present before the acetabulum becomes recognizable as such. While still in the 4-flame-cell stage, the oral sucker appears and there is a solid column of cells which subsequently develops into the intestine. At this stage, neutral fibers are easily recognizable in the anterior part of the cercaria (Fig. 10) and slightly enlarged cells, the precursors of penetration glands, are distinguishable from surrounding body cells. A lumen is present in the intestine, penetration glands have developed short ducts, and accumulations of pigment appear lateral to the pharynx by the time of formation of the third and fourth pairs of flame cells. Posteriorly, the body mass separates into body and tail regions with primary excretory ducts opening laterally, and the mesodermal mass, the forerunner of the excretory bladder is present in the region in which the excretory ducts fuse to initiate bladder formation. The latter process follows essentially the pattern as described by one of us (REK, 1952) for an acanthostomatid, *Cercaria coronanda.* A dilatation at the body-tail junction develops into the excretory atrium, which becomes more apparent in the mature cercaria. The vitelline membrane is present on all specimens up to this time and often persists until near maturity of the cercaria within the redia.

Subsequent development of the cercaria involves rapid growth and elongation of the tail and differentiation of all systems within the body. Characteristic membranes and tail fins, body pigment and numerous other details do not reach their final status of development, or in the case of the acetabulum, degeneration, until the cercaria has escaped into the body tissues of the snail.

Seven pairs of large, crenate penetration glands, numerous cystogenous cells and varying quantities of pigment throughout the body of the cercaria nearing

maturity make study of the excretory system, recognition of the degenerative acetabulum and collapsed intestine difficult to observe. Accurate measurement of some organs is impractical.

The cercaria is biocellate and of the pleurolophocercous type. Measurements of the mature cercaria (fixed in hot 10 per cent formalin) in microns are as follows (mean measurements in parenthesis). Body length, 176 to 224 (205); greatest body width, 68 to 81 (76); tail length, 471 to 513 (493); greatest width of tail, 26 to 29, (27); length of oral sucker, 33 to 48, (41); width of oral sucker, 26 to 33 (30).

The body is covered with a well formed cuticle which anteriorly extends into the oral sucker and posteriorly into the lips of the definitive excretory pore. Dorsally, there are 3 areas of spination (Fig. 21). Anteriorly, the spines are rather evenly distributed and more prominent than in other areas. There are several rows of slightly larger spines in an area anterior and dorsal to the opening of the oral sucker. Spines decrease in number and size posteriorly, the area of spination extending farther laterally (Fig. 21, area *a*). In the mid-part of the body, there is an area with very few or no spines (area *b*) and posteriorly, there are a few irregularly scattered spines, the number being greater at the posterior tip of the body.

The oral sucker is moderately well developed and communicates with the intestine which posterior to the pharynx may be collapsed, relatively degenerate, and seemingly absent in many of the mature cercariae. An accumulation of cells or a mucoid-like mass is present in the posterior lumen of the oral sucker in some specimens. In living cercariae, 2 or 3 pairs of cells which appear to be glandular may be seen in the ventroposterior part of the oral sucker (Fig. 18). Ducts of the penetration glands, apparently cuticularized at their outer ends, open anteriorly and above the cuticularized lips of the opening of the oral sucker. Posteriorly, the cell remnants of the degenerative acetabulum may be seen in some cercariae, but are absent in most. When present, it appears as a mass of cells lying below the surface. Fundaments of reproductive organs are located between the acetabulum and the excretory bladder, but are apparent only in the clearer specimens.

The 7 pairs of penetration glands occupy a considerable part of the cercaria and frequently obstruct view of the internal organs. Ducts of the 4 posterior gland cells pass anteriorly as a group nearer the midline of the cercaria while the other group of 3 ducts is more lateral. Cystogenous cells of varying size move about in the parenchyma tissues of the entire body. Pigment cells and free intercellular pigment granules of varying shades of yellow and brown also move around in the body parenchyma. The eye spots are deeply pigmented, cuboidal, and surrounded by diffuse noncellular pigment.

The excretory bladder has heavy cellular walls and varies in shape, depending on the state of contraction. When filled with fluid, the main part of the bladder is roughly globular with 2 rounded, antero-lateral bulges. From the dorsal aspect, the bladder may appear bilobed. Primary collecting ducts are dilated and receive the anterior and posterior secondary ducts in the mid part of the body at the level of the bifurcation of intestine. At no stage in development of cercariae were ciliated patches observed in the dilatation or elsewhere in excretory ducts. Each secondary duct carries fluid from 3 sets of flame cells (Fig. 16). There is a total

of 36 flame cells and the excretory pattern is expressed as $2[(3+3+3)+(3+3+3)]$. Posteriorly, the excretory bladder possesses well developed lips which conduct excretory fluids into the excretory atrium. Remnants of the caudal excretory tubes appear in the anterior part of the tail. During the process of fluid expulsion, and when partly contracted, the posterior end of the bladder appears thicker than at other times and columns of contracted tissue project inwardly (Fig. 19). Two structures of unknown origin and function were observed in the posterior region of several decaudated cercariae. These appeared as large cells with a vacuole (Fig. 20).

In the mature cercaria, the tail is over twice the length of the body. A prominent clear membrane with slightly thickened supports at irregular intervals extends midventrally and middorsally along the entire length of the tail and may fan out in irregular shapes beyond the posterior tip. The vertical measurement of the caudal membrane varies. It is wider a short distance posterior to the body and again in the distal quarter of the tail. Circular, oblique, and longitudinal muscles are apparent in the tail and rather prominent semi-fixed nucleated cells occur in the parenchyma. The anterior end of the tail is tapered and inserted into a cavity at the body-tail junction. In life, the movements of the body and its pendent position in water are governed in great part by the semi-buoyant nature of the tail. The broad caudal membrane causes the majority of fixed specimens to come to rest on their sides.

## DISCUSSION

The general pattern of development of trematodes, although variable, has been recognized as a characteristic of family or larger groupings. Opinions on the redial potential and germinal lineage of trematodes vary considerably and in some cases are contradictory. Persistence of mother rediae, a feature observed by Cort et al (1948) in a study of representatives of the Fasciolatoidea, and the continued production of daughter rediae of *H. aequalis* in naturally infected *Pirenella conica* has presented opportunity to observe some of the earlier stages of development. Although the presence of both cercariae and daughter rediae in the same mother redia is not considered as a part of the regular course of events in trematode development, Looss (1896) found this condition in *Gastrodiscus aegyptiacus* and Beaver (1939) noted its occurrence in a snail with an old infection of *Psilostomum ondatrae*. The fact that this relationship was observed in only 6 of several hundreds of rediae of *H. aequalis* would seem to indicate that this means of multiplication is not common or may possibly have been induced by the adverse conditions under which the hosts had lived.

The works of Cort (1948, 1954) and his colleagues have emphasized the importance of a knowledge of the development of germinal elements, persistence of centers of multiplication and methods for production of subsequent generations of embryos for an understanding of the basic relationships among the trematodes. Usually germinal cells and embryos develop from specialized cells localized in the parent germinal sac. The development of embryos, as a rule, occurs in the loose tisues in the posterior end of the body cavity, and one cannot predict whether these embryos in the early undifferentiated state are destined to develop into cercariae or daughter rediae. With few exceptions, rediae are of 2 types: those that produce additional generations of daughter rediae, and others that give rise only to cer-

cariae. Cort *et al* (1948, 1954) have shown that the manner of production of germinal cells and masses in rediae or cercariae varies from species to species or even from one redial generation to the next in the same species.

Two separate and well defined sites for production of germinal cells, as observed in *H. aequalis,* introduce an interesting point of speculation with regard to the germinal development of trematodes. Observation of the formation of germinal material at 2 sites in the redia is not entirely new. Cort *et al* (1948) have shown that germinal masses consisting of unicellular and multicellular components may occur in the lateral as well as posterior walls of the body cavity in the mother rediae of *Notocotylus urbanensis* and *Psilostomum ondatrae.* They further called attention to the fact that the germinal mass appeared in 2 separate parts in the former. Possibly the condition of separate sites of embryo production in *H. aequalis* is related to that observed in *N. urbanensis.* However, the nature of the germinal elements at 2 centers is quite different in *H. aequalis,* and as already indicated, has resulted in the presence of both cercariae and daughter rediae in the same germinal sac. While the mother rediae containing both types of embryos of *H. aequalis* are admittedly few, the observation that rediae can have their origin in the lateral walls of the body cavity, and cercariae appear to develop only in the posterior station, suggests the possibility of 2 different types of germinal cells. Certainly this relationship indicates a persistence of germinal centers and does not parallel the simple production of rediae as recognized by Ameel *et al* (1950) in their discussion of the development of *Cryptocotyle lingua* and *Euryhelmis monorchis.* Obviously additional studies are desirable to learn how common this type of embryo-production may be and whether it can be considered as a basic characteristic of the heterophyid trematodes.

A study of daughter rediae reveals the presence of prominent gland cells which seem to be associated with the intestinal tract. This type of cell has been reported only infrequently in the life history and development studies of the trematodes. Cort (1915), Bennett (1936) and Bennett and Humes (1939) have described similar cells in amphistome rediae and Lundahl (1941) found small, unicellular penetration glands in the immature rediae of *Caecincola parvulus.*

Although a number of cercariae of the pleurolophocercous and closely related types have been described, those for specific trematodes of the family Heterophyidae are few. Sonsino in 1892 described *Cercaria pleurolophocerca* from a freshwater snail in Egypt. Khalil (1932) associated this cercaria with a new species of heterophyid, which he designated as *Qasr ainy.* He also worked out the life cycle of *Heterophyes heterophyes* and included a brief description of the cercaria. Although difficult to compare, these cercariae are grossly similar to the cercaria of *H. aequalis.* Recently Martin and Kuntz (1955) described the cercariae of *Stictodora tridactyla* and *Heterophyes* sp. from *Pirenella conica* taken at the same locality from which we obtained *H. aequalis.* These cercariae are morphologically similar, as also are the cercariae of *Monorchotrema taihokui* and *M. taichui* (now *Haplorchis pumilio* and *H. taichui*) described earlier in China by Faust and Nishigori (1926). The cercariae of *Metagonimus yokogawai* and *H. aequalis* differ in many respects but it is of interest to note that a figure of the former (Craig and Faust, 1951) shows cupped structures in the posterior end of the body which may bear some relationship to the large vacuolated cells shown for *H. aequalis.*

Descriptions of heterophyid cercariae indicate a certain homogeneity in some of the basic features but not in others. These cercariae are similar in general appearance, body and oral sucker spination, and the majority are characterized by 7 pairs of prominent penetration glands. Disposition of the latter ranges from a lateral linear arrangement in some cercariae to a mid-central grouping in others. A ventral sucker, if present, as in *H. aequalis,* is poorly developed. It is seen only with difficulty in some species (*H. heterophyes* and *Qasr ainy*) and is absent in others (*Stictodora tridactyla*). The basic plan of the excretory system is variable and unfortunately the flame cell pattern is not known for all species. However, the formula $2[(3+3+3)+(3+3+3)]$ applies to the cercariae of *Heterophyes* sp. and *Stictodora tridactyla* studied by Martin and Kuntz (1955) and to *H. aequalis.* This is not in agreement with the pattern of $2\ (3+7+7)+(7+7+7)$ for the metacercaria of *Cryptocotyle lingua* and $2\ (3+3)+(3+3)$ for *Heterophyes* as reported by Looss (Stunkard, 1929), $2(2+2+3+2)$ for the cercaria of *Euryhelmis monorchis* (Ameel, 1938) or that of $2\ (2+2)+(2+2)$ for a supposedly related acanthostomid (Kuntz, 1952). Obviously, there is need for additional information to determine the relative position of species with modified flame cell formulae and to learn whether or not there is a basic plan of the excretory system in the Heterophyidae.

The general plan of development and morphogenesis of organ systems in the cercaria of *H. aequalis* is basically similar to those described previously in other cercariae of the pleurolophocercous type (Hussey, 1941; Kuntz, 1952) and in the pleurolophocercous cercaria of *C. parvulus* by Lundahl (1941). In the cercaria of *H. aequalis,* as in those considered in previous studies of embryonic development, numerous cystogenous cells and intercellular pigment in the body interfere drastically with observation of some of the internal organs. In each study, emphasis has been given to the formation of a conspicuous, thick-walled excretory bladder derived from a mesodermal mass.

## SUMMARY

The embryonic development of the redial and cercarial stages of *Heterophyes aequalis* has been followed in materials obtained from the snail, *Pirenella conica.* Naturally infected *Pirenella* were collected from the brackish water of Lake Burullus in Lower Egypt. Adult flukes for identification were obtained from laboratory infections of *Gambusia affinis* which served as the second intermediate host, and the subsequent feeding of metacercariae teased from the musculature of this fish to laboratory-reared kittens.

The unusual occurrence of daughter rediae and cercariae in a few mother rediae permitted study of the early developmental stages. Two separate and well defined sites for the production of redial and cercarial embryos suggests the possibility of 2 different types of germinal cells. A study of young daughter rediae revealed the presence of prominent glandular cells which have been observed previously in only a few species of trematodes. Cercarial development is similar to that described by other investigators for cercariae of the pleurolophocercous type with a prominent thick-walled excretory bladder. The cercaria of *H. aequalis* is described.

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## Explanation of Plates

(Figures drawn free hand, majority from living materials.)

Abbreviations: *a*, acetabulum; *bp*, birth pore; *bpo*, birth pouch; *cc*, cystogenous cells; *ce*, cercarial embryos; *cm*, cells of mesodermal mass; *cum*, cercarial vitelline membrane; *dep*, definitive excretory pore; *do*, openings of gland ducts; *e*, pigmented "eye" spots; *ea*, excretory atrium; *eb*, excretory bladder; *ed*, excretory duct; *esp*, esophageal plug; *fc*, flame cells; *gc*, gland cells; *gd*, gland ducts; *gmc*, germinal cells; *i*, intestine; *mc*, muscle cells; *n*, nucleus; *nf*, nerve fibers; *os*, oral sucker; *p*, pigment; *pep*, primary excretory pore; *pg*, penetration glands; *ph*, pharynx; *r*, redia; *rct*, remnants of caudal excretory tube; *s*, surface structure; *vc*, vacuolar cells.

## Plate I

Figure 1. Posterior part of mature mother redia with sites of production for redial and cercarial embryos.

Figure 2. Part of lateral wall of mother redia showing germinal cells and developing redial embryos.

Figure 3. Early daughter redia with well developed intestine and gland cells; germinal cells located posteriorly.

Figure 4. Anterior end of redia showing well developed pharynx with finely-spined, cuticularized lining.

Figure 5. Older actively productive daughter redia with numerous cercarial embryos in body cavity.

Figure 6. Early stage cercaria attached by peduncle to posterior wall of body cavity.

## Plate II

Figure 7. Older cercaria after appearance of flame cells.

Figure 8. Young cercaria with two pairs of flame cells; early differentiation of pharynx.

Figure 9. Older cercaria; early differentiation of acetabulum.

Figure 10. Later stage with two pairs of flame cells but moderately well developed intestine, represented by columns of cells without lumen; early penetration glands and nerve fibers recognizable.

Figure 11. Cercaria with early pigmented "eye" spots; excretory tubes in region of mesodermal mass prior to fusion.

Figure 12. (also Fig. 11; and Fig. 15 of PL. III) Metamorphosis of mesodermal mass and fusion of excretory ducts to form excretory bladder.

Figure 13. Mature mother redia showing excretory system, the birth pouch and sites of development for daughter rediae and cercariae.

Figure 14. Contracted walls of birth pouch during "birth" of cercaria.

## Plate III

Figure 15. Posterior end of cercaria. Excretory tubes fused and surrounded by loose mass of cells; excretory atrium well developed.

Figure 16. Mature, naturally emerged cercaria.

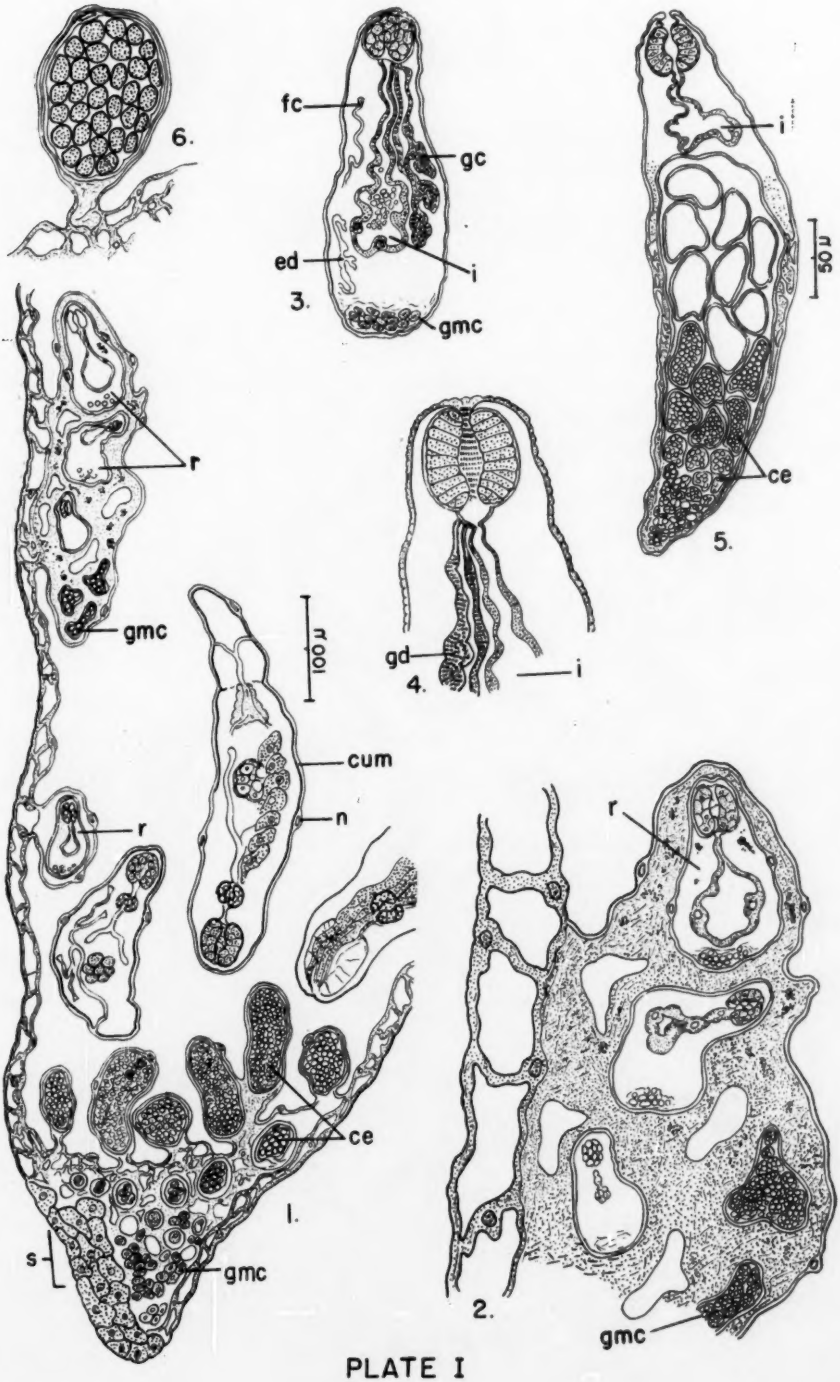
Figure 17. Lateral view of mature cercaria.

Figure 18. Lateral view of anterior end of cercaria showing openings of ducts from penetration glands, esophageal plug and glandular cells in oral sucker.

Figure 19. Lateral view of posterior end of mature cercaria showing excretory atrium and body-tail relationship; excretory bladder partly contracted.

Figure 20. Posterior end of decaudated cercaria showing definitive pore and large vacuolated cells.

Figure 21. Outline of body indicating areas of spination on dorsal surface of cercaria: (*a*) anterior area with more prominent and evenly distributed spines; (*b*) middle area with spines irregular in distribution and size; few in number; (*c*) posterior area in which spines are more numerous than in area *b* but less than in area *a*; number and distribution irregular.

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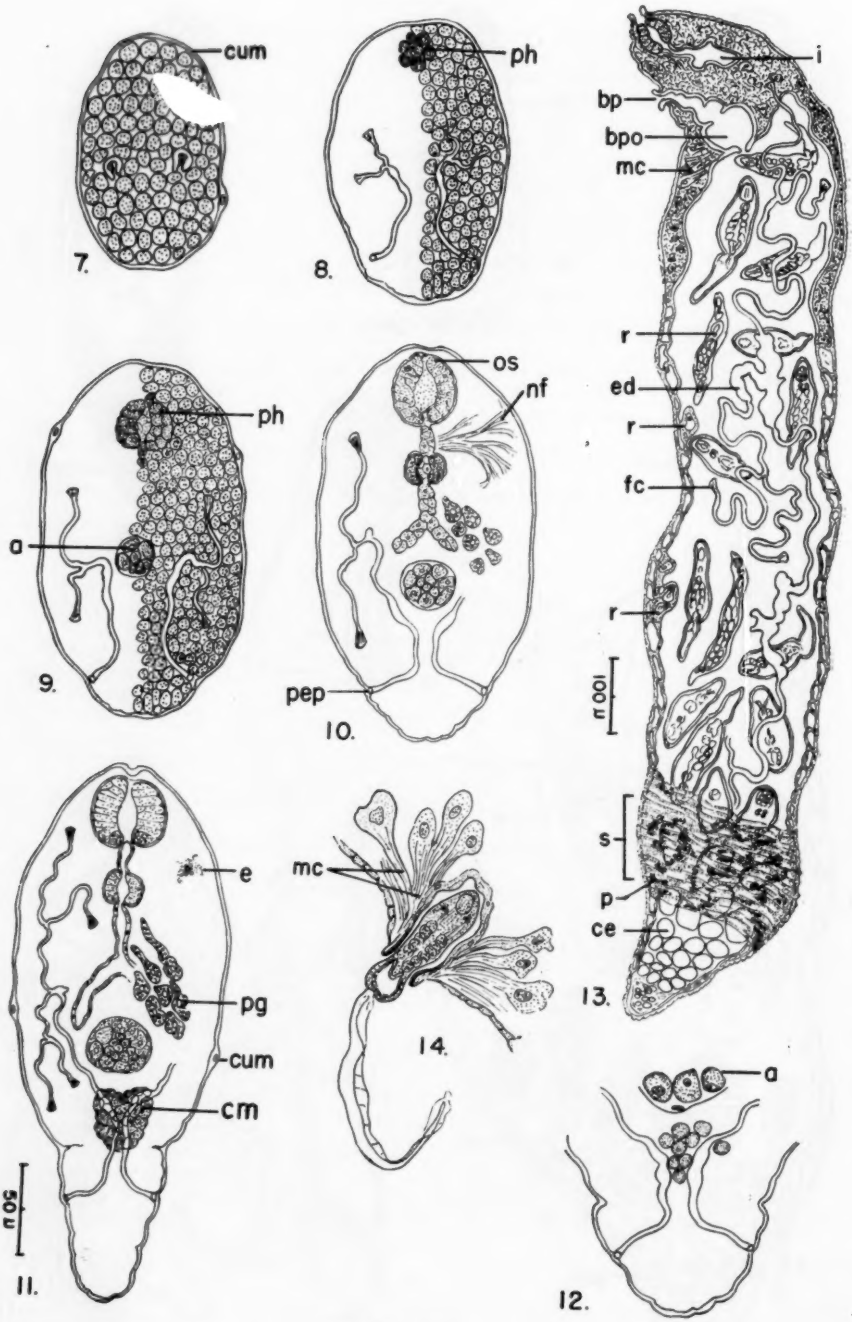
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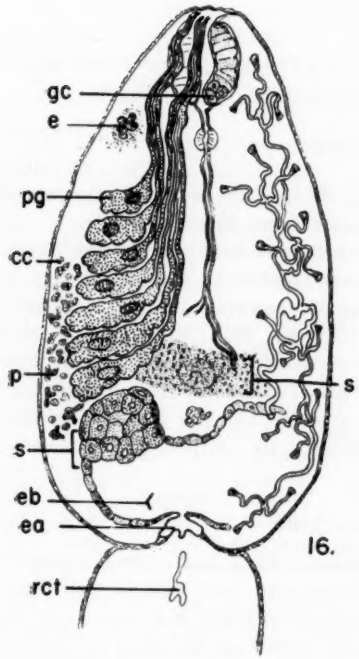


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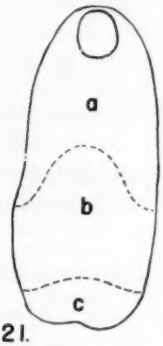


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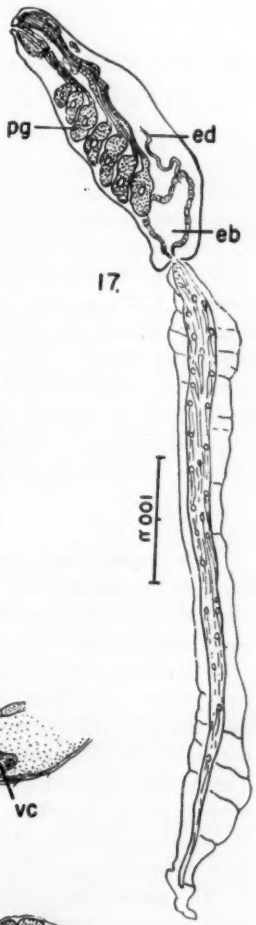


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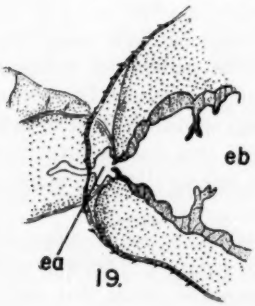


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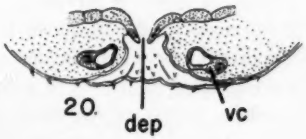


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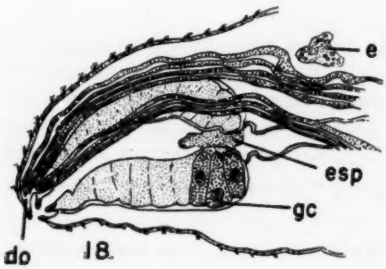


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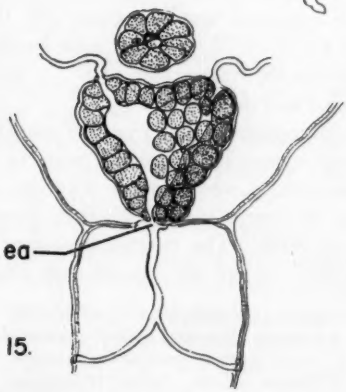


15.

PLATE III

# MORPHOGENESIS OF THE PARASITIC STAGES OF *OSTERTAGIA OSTERTAGI*, A NEMATODE PARASITE IN CATTLE*

FRANK W. DOUVRES†

*Ostertagia ostertagi* (Stiles, 1892) Ransom, 1907, commonly known as the medium stomach worm, is a trichostrongylid nematode found in cattle.

Observations on the life history of this nematode have been made by Stadelmann (1892) and Threlkeld (1946). However, certain aspects of its parasitic development are not adequately described in these authors' reports. Since several investigators (Threlkeld and Bell, 1952; Bailey and Herlich, 1953; Bailey and Thorson, 1954; and Vegors, *et al*, 1955) recently have reported that in epizootics of cattle parasitism, large numbers of immature specimens of this and other nematodes are encountered at autopsy, it appeared necessary to restudy the parasitic developmental stages of *O. ostertagi* with special reference to the morphological features of value in their identification.

## MATERIALS AND METHODS

Five parasite-free, grade Jersey calves obtained from local dairies within 24 hours of birth were used. They were raised in portable pens (Davis, 1949) until infected, at which time they were moved to individual stalls in a concrete-floored barn, and maintained in the manner described by Porter and Cauthen (1942).

The calves were given orally, by means of a blowpipe attached to a 5-cc rubber bulb, either 1 or 2 doses of infective larvae of *O. ostertagi* and were slaughtered at the intervals after infection shown in Table I. For the recovery of worms from the calves at post-mortem, the methods described by Porter (1942) were used. The larvae used were isolated by means of a Baermann apparatus from sphagnum-moss cultures (Cauthen, 1940) of the feces of a calf that had an experimental *O. ostertagi* infection induced by administering to it infective larvae reared from eggs removed from identified females of this species.

To confirm the purity of the cultures, 60 heat-killed, or iodine-killed, larvae were measured and compared with the descriptions given by Threlkeld (1946) and Keith (1953). Since both freshly isolated larvae and larvae that had been stored in the refrigerator (4.5° C) for 1 to 2 weeks were used, some of each were studied and measured.

All measurements given in Table II and in the text were made from heat-killed, unstained specimens floating freely in 0.85% saline under the coverslip to prevent distortion. Camera lucida drawings were made from specimens so treated and from specimens killed with 10% formalin, cleared with lactophenol and mounted in glycerine. The internal anatomy was also observed in specimens killed with hot 70% alcohol and cleared with glycerine, to which a small amount of alcoholic

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eosin-Y had been added. *En face* preparations were made according to the method described by Buhrer (1949).

## RESULTS

### *Preparasitic third-stage larva*

All of the larvae examined morphologically agreed with Keith's (1953) and Threlkeld's (1946) descriptions of the infective, third-stage larva of *O. ostertagi;* however they were slightly longer than the specimens examined by these authors. The heat-killed and iodine-killed larvae which had been refrigerated, averaged, including the sheath, 32 and 51 microns longer, respectively than the freshly Baermannized non-refrigerated, heat-killed larvae.

### *Parasitic stages*

The approximate time required for development to each of the parasitic stages is given in Table I.

TABLE I—*Data on experimental infections with* Ostertagia ostertagi *in 5 calves and parasitic stages recovered following slaughter*

| Calf no. | Larvae per dose | Total larvae given | days from administration of dose(s) to slaughter | Parasitic stages predominantly recovered |
|---|---|---|---|---|
| 1 | 16,500 | | 2 | Third |
| | 16,500 | 33,000 | 4 | Early fourth |
| 2 | 48,000 | 48,000 | 3 | Third molt |
| 3 | 50,000 | | 7 | Late fourth |
| | 50,000 | 100,000 | 12-2/3 | Fifth |
| 4 | 30,000 | 30,000 | 7-5/6 | Late fourth<br>Fourth molt |
| 5 | 25,000 | 25,000 | 10 | Fourth molt |

Larvae in all stages of development and immature adults alike were recovered exclusively from the abomasal mucosal scrapings and washings; none was recovered from the abomasal contents. Their presence in the abomasal mucosa was confirmed by examination of stained histological preparations.

The textual descriptions are primarily anatomical and include only those measurements not given in Table II. Unless otherwise noted, the anatomy is described as observed in median optical section of toto-mount specimens under low (16-mm and 8-mm) and high dry (4-mm) microscope objectives.

*Parasitic third-stage larva.* The third-stage larva resembles the corresponding larva of related trichostrongylids; the body is slender and cylindrical for most of its length and tapers more posteriorly than anteriorly.

The head consists of 3 prominent lips, bearing barely discernible cephalic papillae and 2 very prominent lateral amphids (Fig. 1). The mouth opens into a medially located, tubular buccal capsule, 5 to 7 microns long, which has thin, refractive walls, is conical at its base, and is partially surrounded by the esophagus. Projecting anteriorly from the tip of the claviform esophagus are 2 laterally situated processes, which appear to be extensions of esophageal tissue (Fig. 1). The intestine is composed of 16 elongate nucleated cells, and is followed posteriorly by a slender rectum.

The only discernible components of the excretory system are the excretory pore, which opens into a terminal duct averaging 15 microns long, and 2 excretory gland cells, averaging 25 and 32 microns long. The genital primordium consists of 2 adjoining, oval, or elliptical bodies whose combined length is 14 microns. The tail is often bent slightly dorsad and is dactylate due to an abrupt indentation at an average distance of 10 microns from its tip. This indentation occurs only ventrally, the tail being perfectly straight dorsally, and is better observed in heat-killed than in formalin-killed specimens (Fig. 5).

*Larva in the third molt.* Larvae in this stage are enclosed in a sheath which is easily seen at the cephalic and tail ends. The head structures do not differ critically from those of the third-stage larva. Under an oil immersion (2-mm) objective, the buccal structures appear as follows: In the sheath, the walls of the tubular buccal capsule are represented by 2 thin, highly refractive lines, extending for a distance of approximately 6 microns from the cephalic tip (Fig. 2); at the cephalic tip of the larva proper, the buccal walls are abruptly thickened, appearing as 2 short, sclerotized, rod-like bodies, 1 micron long; caudad to these, the walls again appear as 2 thin, refractive lines (Fig. 2). The base of the buccal capsule is cone-shaped (Fig. 2). The posterior portion of the buccal capsule is surrounded by the esophageal corpus, which is truncate anteriorly and differentiated into 2 regions: A very short, anterior, less granular and almost hyaline portion, demarcated by a thin refractive line from a relatively long, posterior, more dense portion (Fig. 2).

At the level of the junction of the anterior and posterior divisions of the corpus of the esophagus are 4 highly refractive, ovoid structures (Fig. 2), which in later stages proved to be cuticular modifications.

The intestine, compared to that of the third-stage larva, is characterized by a lack of cellular definition, an increase in the number of nuclei and a wider lumen. The excretory gland cells terminate posterior to the base of the claviform esophagus, one cell more posteriorly extended than the other. The length of the cells, based on the distance from the excretory pore to their respective tapering, rounded, posterior ends, averages 377 and 421 microns. The genital primordium appears as a single elliptical and multicellular body. Its distance from the anus falls into either one or the other of 2 restricted, widely separate ranges (Table II); individuals in which this distance falls in the lower range are females, whereas the others are males, since in the next stage when definite anatomical criteria of sex appear, the genital primordium is closer to the anus in females. The tail of the sheath is dactylate, while the tail of the larva proper is bluntly rounded at its tip (Fig. 6). The dactylate tail of the sheath is better observed in heat-killed than in formalin-killed specimens.

When the larvae exsheath, the anterior tip of the discarded sheath bears impressions of all of the cephalic and buccal structures seen in sheathed and unsheathed larvae of this stage.

*Fourth-stage larva.* The head structures, lips, papillae, and amphids, are observed with much difficulty in the 4-day-old larvae, but are better observed in the older ones (Table I). A circular mouth opens into a cuticularized, conical buccal capsule. Anteriorly, the walls of the buccal capsule are much thickened and appear as 2 rod-like, or tooth-like (under 2-mm objective), structures, located between the cephalic tip and the anterior tip of the esophagus; posteriorly, they appear as 2

thin, refractive lines, completely surrounded by esophageal tissue (Figs. 9 and 3). The portion of the buccal capsule surrounded by esophageal tissue is longer in the youngest, than in the older larvae (Figs. 3 and 4).

The corpus of the esophagus consists of 2 histologically differentiated regions: An anterior, strongly muscular portion, averaging 3 to 5 microns in length, recognized by the presence of strong, diagonal striations, and a clearly demarcated, posterior, lightly transversely striated portion (Figs. 3 and 4). The demarcation line between the 2 divisions of the corpus can be seen with the high dry objective, whereas the diagonal striations of the anterior portion are more readily discerned with the 2-mm objective. Superficially, at the level of the terminal end of the diagonally striated portion of the corpus of the esophagus, are seen 4 ovoid or C-shaped structures (Fig. 4). They appear to be cuticular modifications.

Sex is readily distinguishable in the youngest larvae (Table I). In the males, the genital primordium, which is relatively far removed from the anus (Table II), appears as a double row of cells, usually tapers posteriorly and is claviform. There is an abrupt widening of the body at the level of the anus in males. The lengths of the 2 excretory gland cells are 536 and 596 microns and 497 and 575 microns, in the males and females, respectively. In the females, the genital primordium, its posterior end being relatively close to the anus (Table II), consists of numerous cells which extend equidistantly anteriorly and posteriorly from a primitive vulva (Fig. 8). The body of the females is not expanded at the level of the anus (Fig. 8). In both sexes, the tail tapers to a smoothly rounded end (Fig. 8); the tip of the tail in some specimens appears knob-like.

The older larvae are not only larger (Table II) than the youngest ones, but differ from them in having a thicker cuticle with prominent, regularly spaced, transverse striae and more mature reproductive systems. In these older females, the vulva, vagina and muscular portions of the ovijectors are distinctly recognizable and extending anteriorly and posteriorly from the latter structure are numerous cells incipiently oganized as primitive uteri and ovaries. Spicular Anlagen are seen for the first time in the oldest males (Table II).

*Larva in the fourth molt.* In larvae of this stage, the presence of a sheath is easily discernible in the tail region and at the cephalic end only with a 2-mm objective (Figs. 7 and 10).

The head, buccal capsule and corpus of the esophagus appear to have the same structures as in fourth-stage larvae, not yet in the molt. However, in both totomount and *en face* preparations viewed under a 2-mm objective, the cephalic and buccal structures (Fig. 10) are seen to resemble those of the fifth-stage worm; cervical papillae are not observed.

In both sexes, the reproductive system is more fully developed and differentiated than in fourth-stage larvae, not yet in the molt. In the females, the cellular structure of the uterus and ovary is now well differentiated; although the vulvar flap characteristic of adult females is absent, the body diameter is maximum at the level of the vulva (Table II). In the males, the spicular Anlagen are very evident and the bursal rays make their first appearance (Fig. 7). In both sexes, the tail end of the larva proper is smoothly rounded, as is the posterior end of the sheath, and the cuticle is thick and transversely striated.

*Fifth-stage or young adults.* In this stage, the head and buccal structures are

TABLE II – *Measurements* of parasitic larvae of* Ostertagia ostertagi

| Stage of development | Third | Third molt | Fourth-stage | | | Fourth molt | Fifth stage |
|---|---|---|---|---|---|---|---|
| Days after infection | 2 | 3 | 4 | 7 | 7-5/6† | 10 | 12-2/3 |
| Total length | 0.92 | 1.06 | 1.18 (1.29) | 2.52 (3.08) | 3.16 (4.13) | 3.97 (4.73) | 4.87 (6.12) |
| Width at base of oesophagus | 0.03 | 0.03 | 0.03 | 0.05 | 0.05 (0.06) | 0.06 (0.07) | 0.07 |
| Width at level of anus | 0.02 | 0.02 | 0.03 (0.02) | 0.05 (0.03) | 0.06 (0.03) | 0.08 (0.04) | ‡ |
| Oesophagus length | 0.20 | 0.26 | 0.28 | 0.43 | 0.55 | 0.62 | 0.69 |
| Distance from posterior end of genital primordium to anus | 0.30 | 0.27 (0.10) | 0.22 (0.11) | - | - | - | - |
| Distance from anus to tip of larval tail | 0.08 | 0.08 | 0.08 | 0.08 (0.10) | 0.09 (0.12) | 0.07 (0.13) | - (0.13) |
| Distance from tip of larval tail to posterior tip of sheath | - | 0.01 | - | - | 0.01§ | 0.03 (0.01) | - |
| Spicule length | - | - | - | - | 0.06♦ | 0.09 | 0.23 |
| Width at level of vulva | - | - | - | 0.05 | 0.06 | 0.09 | 0.10 |
| Combined length of vagina and muscular portion of ovijectors | - | - | - | 0.12 | 0.18 | 0.23 | 0.27 |

*Averages in millimeters for 10 males and/or females, except where otherwise noted: where two averages are given, the one in parenthesis applies to females and the one above it to males; where only one average is given it is based on 20 specimens and there were no differences between sexes, or it pertains to a sexual character.

† 20% of total larval load recovered were in the fourth molt.

‡ Measurements were not made.

§ Based on 4 ensheathed males.

♦ Based on 6 specimens with spicular anlagen.

markedly different from those in all earlier stages, except late phases of the fourth molt.

*En face* preparations reveal the presence of 3 lips, 1 dorsal and 2 subventral, bordering a triangular mouth opening; the medial border of each lip bears 2 papillae, accounting for 6 cephalic papillae of the internal circle (Fig. 11). External and caudal to the lips is an hexagonal-shaped cuticularized structure, each of its 6 sides being separated by a small indentation (Fig. 11). External to the hexagonal structure, located at each of the subventral and subdorsal indentations is a single papilla and at each of the lateral indentations is an amphid (Fig. 11). The sensory organs of the external circle are, therefore, represented by 4 papillae and 2 amphids. External and caudal to all the aforementioned cephalic structures, there are in the cuticle 4 thickened and refractive C-shaped structures, 2 being located subventrally and 2 subdorsally (Fig. 11).

In toto-mount preparations, all specimens show a cephalic cuticular inflation which bears the C-shaped structures just mentioned. Although easily observable in *en face* preparations, the lips, amphids and papillae of the internal and external circles are either discerned with difficulty, or not at all, in toto-mounts. Also observable in the latter preparations is a minute buccal capsule, the wall of which appears as 2 coccoid, sclerotized structures (Fig. 13). As in fourth-stage larvae, the corpus of the esophagus is histologically differentiated into 2 regions: An anterior, heavily muscular portion, indicated by strong, diagonal striations, and approximately 10 microns in length, and a posterior, very lightly tranversely striated portion (Figs. 13 and 14). The anterior portion of the esophageal corpus has a bisected snout-like appearance (Fig. 13). External to the snout-like portion of the corpus, and separated from it by vacuolated areas, are seen 2 refractive, arched, bar-like structures (Figs. 13 and 14). They represent optical cross sections through the wall of the hexagonal indented cuticularized structure visible in *en face* preparations (Fig. 11).

Appearing for the first time and present in both sexes is a pair of cervical papillae shaped like arrowheads, located at sub-equal levels and posterior to the excretory pore (Fig. 14); their average length is 51 microns. The distances from the anterior end to the cervical papillae a[illegible] 282 and 270 microns in females, and 276 and 270 microns in males. In the females, the vulva opens to the exterior, the ovijectors, uteri and ovaries are fully formed and a vulvar flap is evident for the first time (Fig. 12). The lips of the vulva are heavily cuticularized and armed; the anterior lip supports 1 spine or projection, whereas the posterior lip bears 3 (Fig. 12). There are no eggs in the uteri. In the males, the spicules, gubernaculum and bursal rays are fully developed; however, they have not attained mature adult dimensions. The excretory gland cells average one-sixth the total body length in females and one-fourth in males.

*Mature adults.* Mature worms were obtained from a calf killed 21 days after it was infected with larvae of the same origin as the ones used by the writer. Since the diagnostic morphology of the adults has been well described by Ransom (1911), a recapitulation of these morphological details will not be given here.

## DISCUSSION

The results of the present investigation gave evidence of 3 ecdyses within the host, in contrast to the findings of Threlkeld (1946), who observed only 2. He also reported that the parasitic third-stage larva did not appear until the third day after infection; that ensheathed fourth-stage larvae approaching the fifth-stage were recovered by the fifth day of infection; and that the final ecdysis from fourth to fifth stage occurred between 7 and 10 days after infection. The present study revealed that parasitic third-stage larvae were present by the second day of infection; that the fourth-stage larvae began ensheathment 7-5/6 days after infection and were still ensheathed 10 days after infection; and that the fourth or final ecdysis was completed by 12-2/3 days after infection.

Stadelmann (1892) described for *Strongylus convolutus* (= *O. ostertagi*) 2 parasitic larval stages separated by a molt; the first was 1.5 mm long and had 2 strongly developed teeth at or near the mouth opening, and the second was larger and lacked those teeth. Stadelmann's description of the first larva closely parallels the descrip-

tion given in the present paper for the fourth-stage larva; the 2 "teeth" undoubtedly are merely optical cross sections through the anterior portion of the buccal capsule wall of the fourth-stage larva. Although Stadelmann's description lacks detail, the second larva he described may have been a fifth-stage worm; this deduction is based on the writer's observation that the buccal capsule is very minute and much smaller than in the fourth-stage larva.

So far as the writer is aware, the 4 C-shaped cephalic cuticular modifications described for the third- and fourth-stage larvae and fifth-stage worms, the histological modification of the anterior end of the corpus of the esophagus described for the fourth- and fifth-stage worms, and the fact that in the latter stage there is seen on *en face* examination, external to the lips, a heavily cuticularized hexagonally-indented structure, have not previously been reported in *Ostertagia* or closely related nematodes. Some authors have illustrated the buccal capsule of *O. ostertagi* as rather large and with walls that appear as arched bars in cross section, whereas the writer finds the buccal capsule to be minute and interprets presumably identical prominent bars as cross sections through the walls of a distinct structure which is external to the buccal capsule and appears as an indented hexagon when viewed *en face*. This conclusion and the conclusion that in fourth-stage larvae, on the other hand, it is the wall of the provisional buccal capsule which presents a similar bar-like appearance in cross section is based on careful study of cut-off heads rotated so as to permit examination from all aspects.

It is of interest that the distance from the excretory pore to the terminal end of the excretory gland cells was found to be 1/20th of the total body length in the parasitic third-stage larva, two-fifths in the larva in the third molt, one-half in the early fourth-stage larva, one-third in the late fourth-stage larva, and one-fourth to one-sixth in the fifth-stage worm. Similar observations on the excretory system were made in a study of *Nematodirus spathiger* (Railliet, 1896) Railliet and Henry, 1909 by Kates and Turner (1955). Although in the ratio of the length of the excretory system to the total body length, there is an increase during the early parasitic stages, and then a decrease in later parasitic stages, it should be noted that as the larvae grow older the absolute length of the excretory system continually increases. The excretory gland cells had a high affinity for eosin.

## SUMMARY

1. Infective larvae of *Ostertagia osteragi* used for infecting calves in the present study were longer than, but morphologically resembled, those described by other investigators; larvae that had been stored in a refrigerator (4.5° C) were much longer than non-refrigerated ones.

2. Larvae in the parasitic third-stage, third molt, fourth-stage and fourth molt and immature fifth-stage worms were recovered, respectively, 2 days, 3 days, as early as 4 and as late as 7-5/6 days, as early as 7-5/6 and as late as 10 days, and 12-2/3 days, after infection.

3. The stage of development of a parasitic larva of this species can be identified by means of morphologic characteristics that are visible when a properly prepared toto-mounted specimen is viewed under low and high dry microscope objectives.

For the third-stage larva, these characteristics are: Sheath absent; buccal capsule tubular with conical base, thin-walled, its posterior portion surrounded by the

esophageal corpus; tip of latter, with 2 anteriorly directed lateral processes located alongside, but separated from, the anterior portion of the buccal capsule; tail dactylate.

For a larva in the third molt, they are: Sheath present; buccal capsule similar to that of third-stage larva, but extending into the sheath and with wall thickened at anterior tip of larva proper; anterior tip of esophagus truncate; tail-tip of larva proper and of sheath, bluntly rounded and dactylate, respectively.

For a fourth-stage larva, they are: Sheath absent; buccal capsule conical, its wall much thickened anteriorly, appearing as 2 rod-like structures located between cephalic tip and anterior tip of esophagus, and posteriorly, very thin and completely surrounded by esophagus. Corpus of esophagus consists of 2 histologically differentiated regions: Anteriorly, a heavily muscular portion, recognized by the presence of strong, diagonal striations and posteriorly, a clearly demarcated, lightly transversely striated portion; body abruptly widened near anus (male), or rudimentary vulva (female) present.

For a larva in the fourth molt, they are: Sheath present; buccal capsule and corpus of esophagus much as in fourth-stage larva; rudimentary bursa, spicular Anlagen (male) or cellular differentiation of uterus and ovary (female), very evident.

4. Sex was first determined in larvae in the third molt; the distance from the genital primordium to the anus is shorter in females than in males.

5. A cephalic inflation, cervical papillae and a vulvar flap were first observed in fifth-stage worms.

6. Two anatomical features not previously reported in *Ostertagia*, or closely related trichostrongylids, are described, namely, (a) 4 C-shaped thickenings in the cephalic cuticle, first appearing in larvae undergoing the third molt, persisting in subsequent stages, and located on the cephalic inflation in fifth-stage worms; (b) histologic modification of the anterior tip of the corpus of the esophagus, first appearing in larvae undergoing the third molt and persisting in all later stages.

7. The cephalic characteristics of the fifth stage are fully described for the first time and include the presence of a cuticularized peribuccal structure which has an indented-hexagonal shape when viewed *en face*; the 2 arched cuticularized bars seen in a median saggital optical section of the buccal region are considered due to its presence. The cephalic and buccal structures characteristic of fifth-stage worms are present in rudimentary form in larvae in the fourth molt.

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## EXPLANATION OF PLATE I

(Drawn with the aid of the camera lucida)

Figure 1.* Cephalic end of the parasitic third-stage larva, dorsal view.

Figure 2.* Cephalic end of the larva of the third molt, dorsal view.

Figure 3.* Cephalic end of a 4-day-old fourth-stage larva, lateral view. Although there are 4 ovoid or C-shaped cuticular structures present at this stage, only 2 are shown in this figure.

Figure 4.* Cephalic end of a 7-day-old fourth-stage larva, lateral view.

Figure 5. Posterior region of the parasitic third-stage larva, lateral view.

Figure 6. Posterior region of the larva in the third molt, lateral view.

FIGURE 7. Posterior region of a larva in the fourth molt (male) lateral view.

Figure 8. Posterior region of a fourth-stage larva (female), lateral view.

Figure 9.* Anterior region of the fourth-stage larva, lateral view.

Figure 10.* Cephalic end of the larva in the fourth molt, lateral view. Although present at this stage, the heavy muscular striation of the corpus of the esophagus is not shown in the present figure.

Figure 11.* *En face* of the fifth-stage worm.

Figure 12. Vulvar region of the fifth-stage worm, lateroventral view.

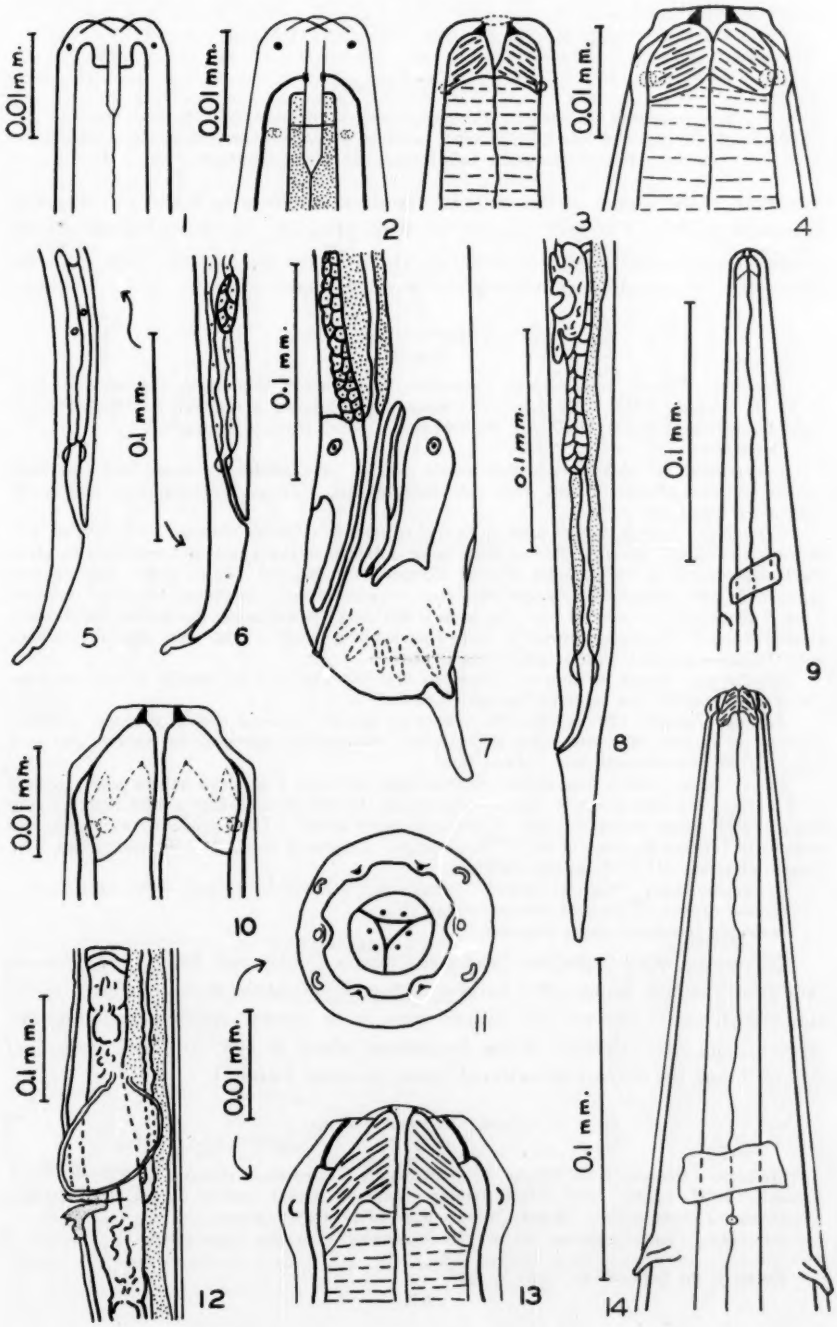
Figure 13.* Cephalic end of the fifth-stage worm, lateral view.

Figure 14.* Anterior region of the fifth-stage worm, showing the location of the cervical papillae, ventral view.

* Drawings made from 10% formalin fixed, lactophenol cleared and glycerine mounted specimens.

All other drawings made from heat-killed specimens, floating freely in saline.

PLATE I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# EIGHT NEW SPECIES OF *IXODES* FROM CENTRAL AND SOUTH AMERICA (ACARINA: IXODIDAE)

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Seven of the 8 new species of ticks described herein were found in collections submitted by the Chicago Natural History Museum for identification. One, *Ixodes chilensis*, was present in material received from the United States National Museum. All measurements are given in millimeters.

*Ixodes guatemalensis* n. sp.
(Fig. 1)

*Holotype.* Female, from Volcan Tajumulco, San Marcos, Guatemala, February 24, 1934. F. W. J. Schmidt, RML 12037. Host: *Sciurus g. griseoflavus*, a squirrel, L. Mandel Guatemala Expedition, CNHM 41667. In the Chicago Natural History Museum.

*Description.*

Unengorged or slightly engorged female. Color of capitulum, scutum, and legs dark brown. Scutum occupies a little more than half the body length. Postscutal area and venter with a few fine hairs.

*Capitulum.* Length, tips of palpi to tips of cornua, 0.74; width of basis, 0.44. Dorsal surface of basis crazed, shining. Porose areas large, subcircular, depressed, and separated by about the distance equal to the diameter of one. Cornua short, pointed. Palpi widest near apex of segment 2; outer margins nearly straight, inner margins curved. Combined length of segments 2 and 3 about 0.51. In ventral view, the basis is flattened, constricted at the middle, and broadly rounded behind. Auriculae distinct as large rounded projections. Transverse sutural line present. Palpal segment 1 with a small ventral plate.

*Hypostome.* Shape as figured. Dentition first 4/4 then 3/3 and finally 2/2 to the base. Teeth of the lateral files largest. Length about 0.41.

*Scutum.* Length, 1.20; width, 0.98. Shape as figured. Lateral carinae distinct. Cervical grooves as shallow divergent lineal depressions. Punctations moderate in number and size, larger in the anterolateral field. Hairs few.

*Legs.* Coxa I with a long slender internal spur and with a posterior salient edge; coxa II with no spurs but with posterior salient edge; coxae III and IV each with a faint external spur and a mildly salient posterior edge. Trochantal spurs absent. Tarsi gradually narrowed subterminally (all but the coxa of leg IV is missing). Length of tarsus I, 0.60; metatarsus, 0.34. Length of tarsus III, 0.48; metatarsus, 0.36.

*Spiracular plate.* Shape as figured. Longer axis transverse. Length, 0.26; width, 0.23.

*Genital aperture.* Situated between coxae IV.

Male and immature stages unknown.

This new species resembles *Ixodes tancitarius* Cooley and Kohls, 1942, known only from a single female off a harvest mouse, *Reithrodontomys* sp., Cerro Tancitaro, Michoacan, Mexico, but differs from it in several respects including the stouter palpi, the dentition of the hypostome which is 4/4, 3/3, 2/2 instead of 3/3, 2/2, and the absence of external spurs on coxae I and II.

*Ixodes tecpanensis* n. sp.
(Fig. 2)

*Holotype.* Female, from Tecpán, Chimaltenango, Guatemala, February 7, 1934; F. W. J. Schmidt. RML 12036. Host: *Orthogeomys grandis*, a pocket gopher, L. Mandel Gutemala Expedition, CNHM 41767. In the Chicago Natural History Museum.

*Paratype.* Female, data as above. In the Rocky Mountain Laboratory.

Received for publication, April 9, 1956.

*Description.*

Female. The two partially engorged specimens are oval, widest at about the middle. Color of capitulum, scutum, and legs, yellow brown. Postscutal area and venter with fine hairs.

*Capitulum.* Length from tips of palpi to tips of cornua, holotype, 0.88; width of basis, 0.55; for paratype, 0.85 and 0.50, respectively. Dorsum of basis mildly convex, and with mild carinae extending from the cornua to the insertion of the palpi. Porose areas large, subtriangular, slightly depressed, well separated. Cornua short, bluntly pointed, posterior margin of basis between them slightly concave. Outer margins of palpi nearly straight, inner margins curved, widest near the apex of segment 2. Combined length of segments 2 and 3 about 0.61. In ventral view the basis is mildly convex and slightly constricted behind the short lobelike auriculae. Sutural line faint. Palpal segment 1 with a small ventral plate.

*Hypostome.* Shape as figured. Dentition 3/3 for about half the length, then 2/2. Lateral denticles conspicuously larger than the medians. Length of toothed portion about 0.43.

*Scutum.* Length and width, holotype, 1.34 by 1.19; paratype, 1.26 by 1.06. Shape as figured. Lateral carinae distinct. Cervical grooves as broad shallow divergent depressions. Punctations sparse and scattered. A few short fine hairs present.

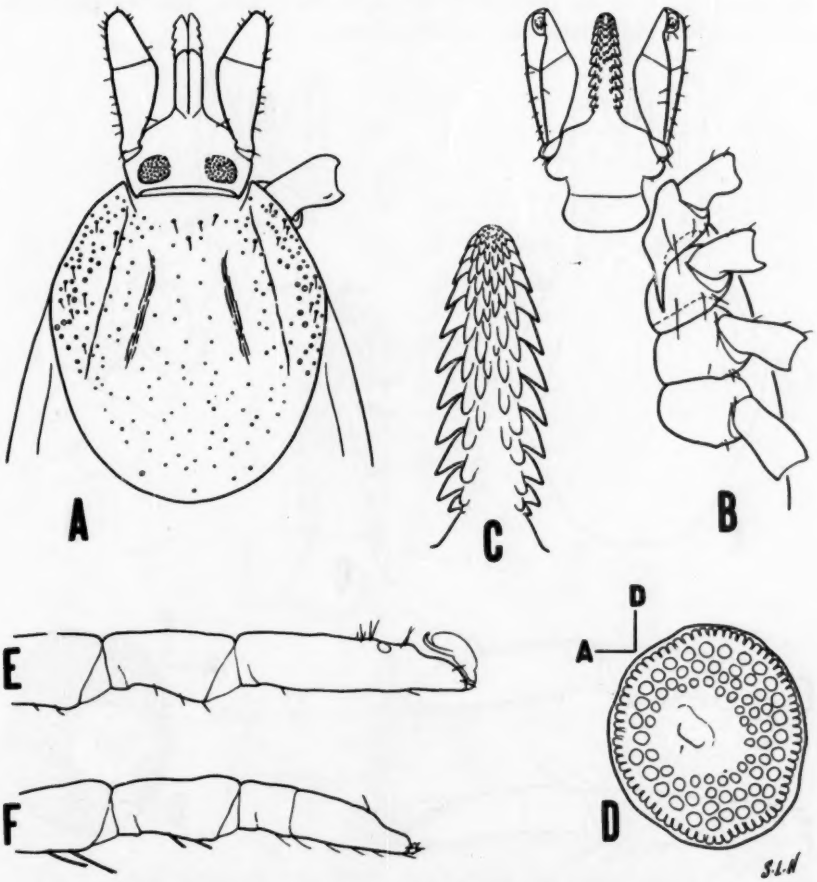


FIG. 1. *Ixodes guatemalensis* n.sp. Female. A. Capitulum and scutum, dorsum. B. Capitulum and coxae, venter. C. Hypostome. D. Spiracular plate (A = anterior; D = dorsal). E. Metatarsus and tarsus, leg I. F. Metatarsus and tarsus, leg IV.

*Legs.* Coxae I to IV each with a short external spur. A long sharp internal spur on coxa I, no internal spurs on coxae II to IV. Trochantal spurs absent. Tarsi gradually narrowed subterminally. Length of tarsus I, 0.67; metatarsus, 0.40. Length of tarsus IV, 0.60; metatarsus, 0.45.

*Spiracular plate.* Shape as figured. Longer axis transverse. Length, 0.32; width, 0.25.

*Genital aperture.* Situated between coxae III.

Male and immature stages unknown.

The characters of the capitulum, scutum, and legs, taken in combination will distinguish this new species from all New World species of this genus. *Ixodes dampfi* Cooley, 1943, described from *Geomys* sp., Chalco, State of Mexico, is the only other species of this genus known from Latin American pocket gophers. In *I. dampfi,* auriculae are absent, the palps are shorter and heavier, the porose areas are smaller and nearly circular, the posterior margin of the basis capituli between the cornua is sinuous and much below the level of the cornua. The two species also differ in details of the hypostome and the scutum.

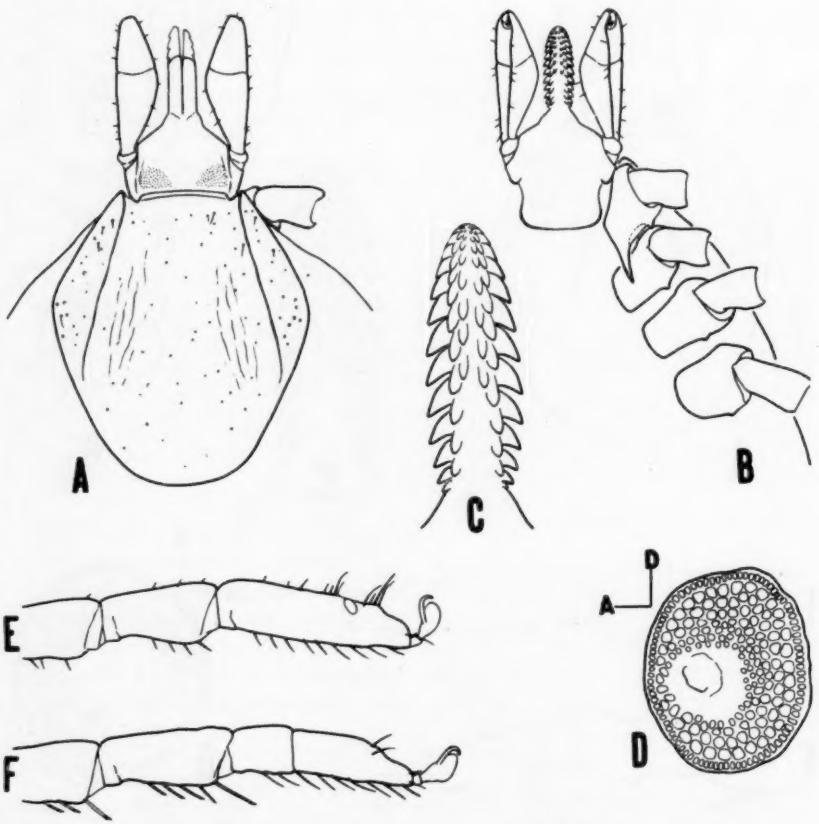


FIG. 2. *Ixodes tecpanensis* n.sp. Female. A. Capitulum and scutum, dorsum. B. Capitulum and coxae, venter. C. Hypostome. D. Spiracular plate (A = anterior; D = dorsal). E. Metatarsus and tarsus, leg I. F. Metatarsus and tarsus, leg IV.

*Ixodes pomerantzi* n. sp.
(Figs. 3 and 4)

*Holotype.* Male, from Canchaque, Huancabamba, Piura, Peru, 1960 meters elevation, May 2, 1954, C. Kalinowski. RML 33575. Host: *Sylvilagus brasiliensis,* a rabbit, CNHM 81194. In the Chicago Natural History Museum.

*Allotype.* Female, data as above. In the Chicago Natural History Museum.

*Paratypes.* Three males, 6 females, data as above; 1 female, locality, host, and collector as above, May 19, 1954, RML 33576, CNHM 81195. One male from Finca San Rafael, Sacatepéquez, Guatemala, 2139 meters elevation, July 1, 1948, R. D. Mitchell and Luis de la Torre, RML 31387, host, *Sylvilagus floridanus chiapensis,* CNHM 64464. Paratypes deposited in the Rocky Mountain Laboratory and the Chicago Natural History Museum.

*Description of male.*

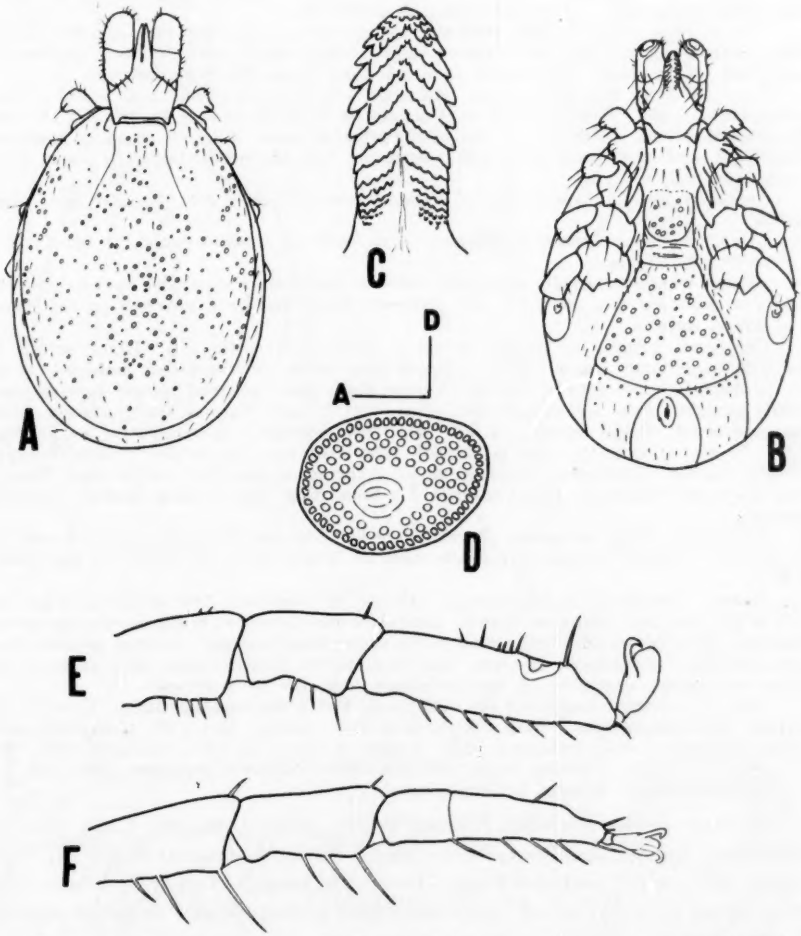


FIG. 3. *Ixodes pomerantzi* n.sp. Male. A, Capitulum and scutum, dorsum. B. Capitulum, coxae, and ventral plates, venter. C. Hypostome. D. Spiracular plate (A = anterior; D = dorsal). E. Metatarsus and tarsus, leg I. F. Metatarsus and tarsus, leg IV.

Length Peruvian specimens, tips of scapulae to posterior margin of body, about 1.31; width, 0.97. Guatemala male about 1.39 by 1.01. Suboval, widest a little behind the middle. Color yellow brown.

*Capitulum.* Length, tips of palpi to tips of cornua, 0.46; greatest width of basis, 0.25. Basis narrowed behind, sides nearly straight. Posterior margin between the cornua slightly convex. Surface of basis with a few punctations. Cornua short, pointed. Palpi moderate in length; segments 2 and 3 about equal, their combined length about 0.30. Ventrally, the basis is broadly rounded and salient posteriorly. Auriculae as blunt lateral saliences at the same level as the ventral surface. Palpal segment 1 with a small, pointed, ventral plate. Several long, fine hairs on ventral edge of palpal segment 2.

*Hypostome.* Shape as figured. Length about 0.26. Longitudinally grooved ventrally and with about 12 diagonal rows of crenulations and about 6 longer lateral denticles on each half.

*Scutum.* Evenly convex except for the anterolateral areas which are mildly depressed; pseudoscutum not well defined. Scapulae short and blunt. Lateral carinae absent. Cervical grooves shallow, divergent. Punctations moderate in size and number, larger and more numerous in the median area. Hairs numerous and fairly long.

*Ventral plates.* Median plate more than twice as long as the anal plate and with large, deep punctations and a few fine scattered hairs. Adanal plates wider in front; punctations fewer and smaller than on the median plate, and hairs longer and more numerous.

*Legs.* Moderate in length and size. All coxae with long, fine hairs. Coxa I with a long, sharp, internal spur. Short external spurs present on all coxae. Coxae II, III, and IV with the internal corners salient and spurlike. No trochantal spurs. All tarsi gradually narrowed distally. Length of tarsus I, about 0.40; metatarsus, 0.22. Length of tarsus IV, about 0.36; metatarsus, 0.23.

*Spiracular plate.* Shape as figured, greatest dimension about 0.20. Longer axis longitudinal.

*Genital aperture.* Situated at the level of the intervals between coxae III and IV.

*Description of female.*

All specimens are partially engorged. Color of capitulum, scutum, and legs dark brown. Hairs on postscutal area short, fine, and scattered; longer and more numerous on the lateral and ventral surfaces.

*Capitulum.* Length, tips of palpi to tips of cornua, 0.78; width, 0.41. Dorsal surface of basis flattened, faintly crazed, shining. Porose areas mildly depressed, well separated, variable in shape but tending to be suboval. Cornua short, blunt, posterior margin between them nearly straight. Palpi widest near apex of segment 2, outer margins nearly straight, inner margins curved. Palpal segment 2 a little longer than segment 3, their combined length about 0.53. In ventral view the basis is strongly constricted near the middle, posterior margin broadly rounded. Transverse sutural line faint. Auriculae as long, thin, curved horns directed downward and backward. Palpal segment 1 a broad ring with a sharp, ventrally directed process.

*Hypostome.* Shape as figured. Dentition 4/4 for about half the length, then 3/3, and 2/2 to the base. Denticles progressively smaller from the lateral files to the middle. Length about 0.46.

*Scutum.* Length and width, allotype, 1.13 by 1.00; paratypes, 1.00 to 1.13 in length by 0.89 to 0.95 in width. Shape as figured. Lateral carinae distinct as broad, rounded elevations extending from the scapular field almost to the posterolateral margin. Cervical grooves shallow, divergent. Punctations numerous and large in the posteromedian field, progressively fewer and smaller anteriorly. A few moderately long, fine hairs present.

*Legs.* Moderate in length and size. All coxae with a few long, fine hairs. Coxa I with a long, thin, internal spur. Each coxa with a short external spur. No trochantal spurs. Length of tarsus I, 0.47; metatarsus, 0.29. Length of tarsus IV, 0.43; metatarsus, 0.29.

*Spiracular plate.* Suboval, longer axis transverse. Greatest dimension about 0.25.

*Genital aperture.* Situated between coxae IV.

The new species resembles *I. tovari* Cooley, 1945, *I. fuscipes* Koch, 1844, *I. spinipalpis* Hadwen and Nuttall (in Nuttall, 1916), *I. dentatus* Marx (in Neumann, 1899), and *I. neotomae* Cooley, 1944. The females of all of these have long, thin, curved auriculae and all but *I. tovari* have a sharp process on palpal segment 1 ventrally.

Males of *I. tovari* are larger and more hairy than those of the new species and the spiracular plate is more elongate. The hypostomes are similar but differ in shape and in the number of diagonal rows of crenulations, there being about 17

of the latter in *I. tovari* and about 12 in the new species. The females are readily separated by the presence in *I. pomerantzi* of the sharp, ventral spur on palpal segment 1. *I. tovari* is known from Mexico and southern Texas and lagomorphs are the principal hosts.

The male of *I. fuscipes* is undescribed. Females at hand from Peru off "agouti" and from Brazil off *Cuniculus paca* resemble *I. pomerantzi* very closely. They differ from it in having the hypostome about 35 percent longer, the denticles arranged 3/3, and in having longer and much more slender palpi.

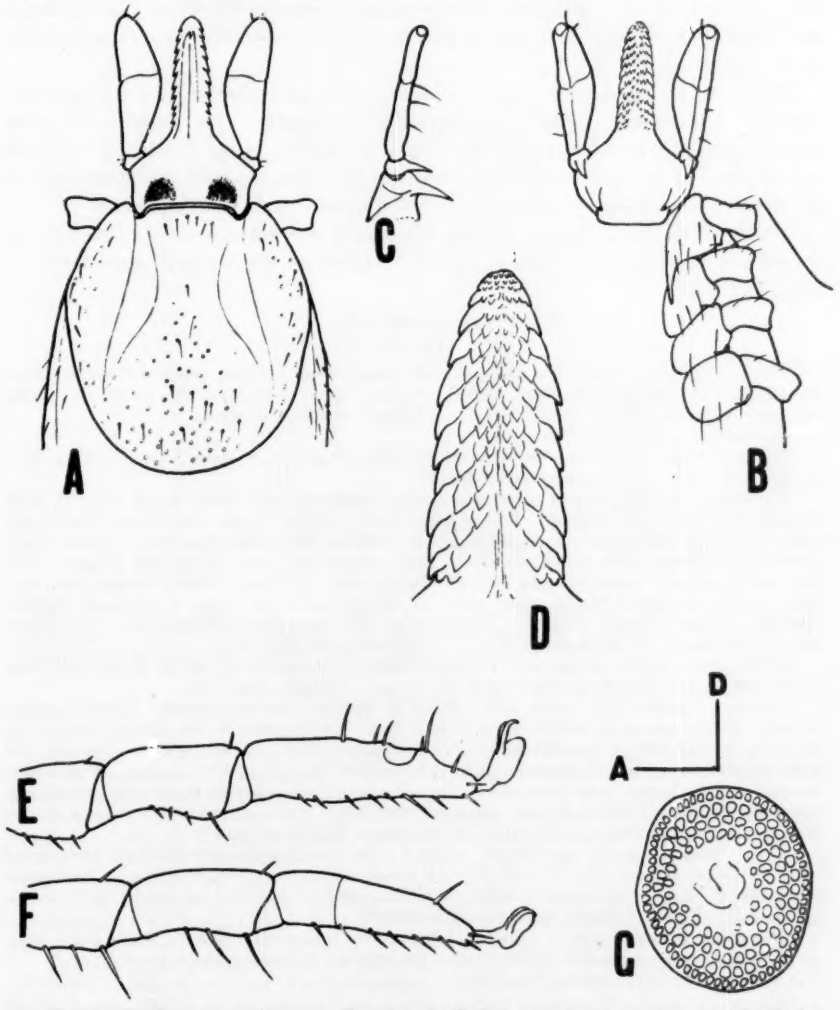


FIG. 4. *Ixodes pomerantzi* n.sp. Female. A Capitulum and scutum, dorsum. B. Capitulum and coxae, venter. C. Lateral view of capitulum. D. Hypostome. E. Metatarsus and tarsus, leg I. F. Metatarsus and tarsus, leg IV. G. Spiracular plate (A = anterior; D = dorsal).

From *I. spinipalpis* the new species is best distinguished by the hypostome of the male. In the former species this structure is mildly notched apically and has only about 4 large lateral denticles and about 6 diagonal rows of crenulations on each half. *I. spinipalpis* is known from western United States and western Canada and it occurs on lagomorphs primarily.

The new species and *I. dentatus*, the latter a rabbit tick of the eastern United States, are easily distinguished by the characters of the female hypostome. In the latter species the hypostome is notably broader and the denticles are arranged 6/6. In the male of *I. dentatus*, the hypostome is narrower than in *I. pomerantzi* and there is much less disparity in size between the lateral denticles and those of the other files.

The males of *I. pomerantzi* and *I. neotomae* may be separated by the hypostome which in *I. neotomae* is similar to that of *I. spinipalpis*. The females are quite similar but in the new species the hypostome is broader, being about 0.18 in width as compared to 0.11 in *I. neotomae*, and the denticles are longer and sharper in the latter. *I. neotomae* is known from rodents and rabbits in California.

The new species is named for Mr. Charles Pomerantz of New York City in recognition of his contribution to studies relative to the medical importance of mites and ticks.

*Ixodes tapirus* n.sp.
(Fig. 5)

*Holotype.* Female, from Río Majuas, 2700 meters, San Agustín, Huila, Colombia, October 21, 1951, P. Hershkovitz. RML 31424. Host: *Tapirus pinchaque*, the woolly, or mountain tapir, CNHM 70557. In the Chicago Natural History Museum.

*Description of female.*

Partially engorged. Color of capitulum, scutum, and coxae, dark brown. Body with sparse, short, fine hairs dorsally and ventrally.

*Capitulum.* Length, tips of palpi to posterior margin of basis, 1.04; width of basis, 0.63. Surface of basis crazed, shining. Porose areas clearly defined, large, subcircular, superficial, and separated by about half the diameter of one. Cornua very short, rounded; posterior margin of basis between them a little concave. Palpi widest near apex of segment 2, outer margins nearly straight, inner margins curved. Palpal segment 2 only slightly longer than segment 3, their combined length about 0.75. In ventral view, the basis is narrower behind, posterior margin broadly rounded. Auriculae as mild ridgelike protuberances. Transverse sutural line faint. Palpal segment 1 with a small ventral plate.

*Hypostome.* Shape as figured. Principal denticles arranged 3/3 in the apical half, then 2/2 to the base. Lateral denticles much the larger. Length about 0.52.

*Scutum.* Length, 1.52; width, 1.52. Shape as figured. Surface shining. Lateral carinae as mild, widely divergent elevations extending from the scapulae to the lateral margins at about the widest part of the scutum. Cervical grooves broad, shallow, and not reaching the posterolateral margins. Anteromedian area between the cervical grooves arched. Punctations unequal in size; larger ones restricted to the anterolateral and lateral fields with a few near the posterior margin; smaller ones scattered over other areas of the scutum, those in the median field minute and inconspicuous. A few short, fine hairs present.

*Legs.* Moderate in size and length. Coxa I with a long, sharp internal spur, no external spur; no spurs on coxae II, III, and IV. All coxae with a few, long fine hairs. Trochantal spurs absent. Length of tarsus I, 0.89; metatarsus, 0.63. Length of tarsus IV, 0.81; metatarsus, 0.63. Tarsi gradually narrowed subterminally.

*Spiracular plate.* Shape as figured. Longer axis transverse. Length, 0.52; width, 0.41.

*Genital aperture.* Situated at the level of the intervals between coxae III and IV.

Male and immature stages unknown.

This new species is readily distinguished by characters of the capitulum, scutum, and coxae, and it does not appear to be closely allied to any other South or Central American species. It is the only species of *Ixodes* thus far recorded from

American tapirs. Associated with it were 4 males of the rare tapir tick, *Amblyomma multipunctum* Neumann.

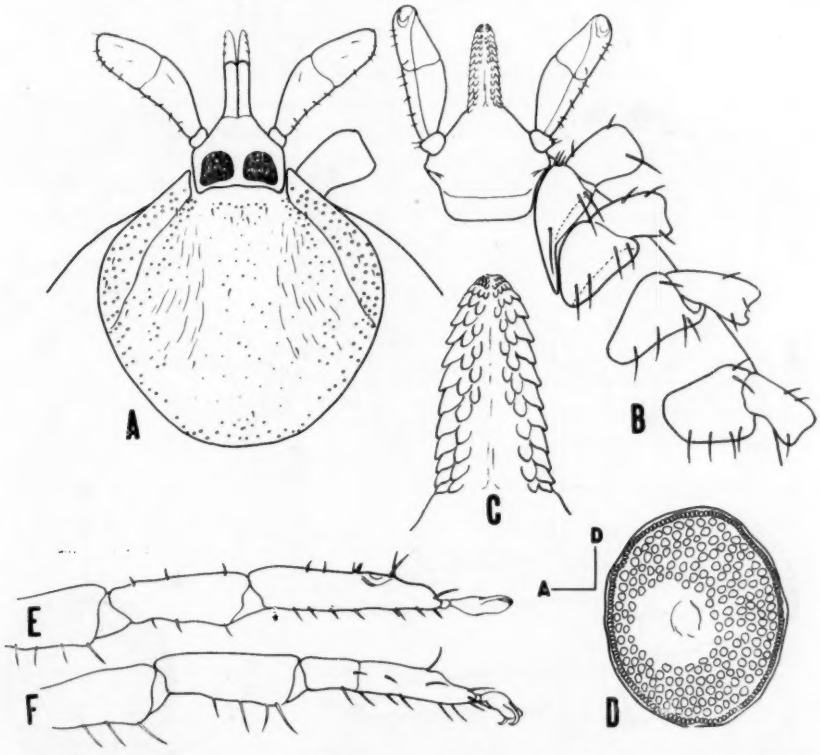


FIG. 5. *Ixodes tapirus* n.sp. Female. A Capitulum and scutum, dorsal. B. Capitulum and coxae, venter. C. Hypostome. D. Spiracular plate (A = anterior; D = dorsal). E. Metatarsus and tarsus, leg I. F. Metatarsus and tarsus, leg IV.

*Ixodes tropicalis* n.sp.
(Fig. 6)

*Holotype.* Female from La Selva, Valdivia, Antioquia, Colombia, 1900 meters elev., July 3, 1950, P. Hershkovitz. RML 31416. Host: *Thomasomys aureus,* a rodent, CNHM 70323. In the Chicago Natural History Museum.

*Paratypes.* Two females from San Juan, Tambopata, Sandia Province, Puno, Peru, November 30, 1950, H. H. Heller. RML 31445. Host: *Dactylomys boliviensis,* a rodent. In the Rocky Mountain Laboratory.

*Description of female.*

All specimens partially engorged. The largest, a paratype, is about fully engorged and is 6.56 long (excluding the capitulum) by 5.13 wide. All are ovoid, and are widest a little behind the middle. Color of capitulum, scutum, and coxae, yellow brown.

*Capitulum.* Length, tips of palpi to tip of cornua, 1.01 for the holotype, and 0.83 for the paratypes. Width of basis 0.48 for the holotype, and 0.42 for the paratypes. Surface of basis finely crazed. Porose areas large, a little depressed, and separated by about the diameter of one. Cornua short and rounded, the posterior margin between them slightly concave. Palpi widest near apex of segment 2, outer margins nearly straight, inner margins curved. Palpal segment 2 only a little longer than segment 3; their combined length, 0.76 for the holotype,

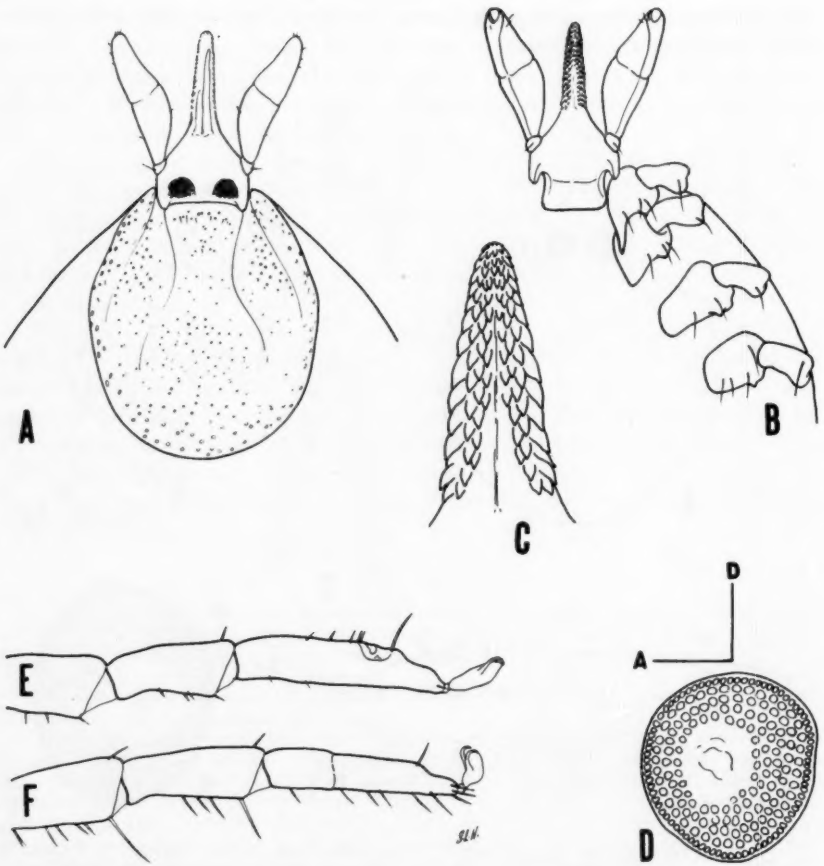


FIG. 6. *Ixodes tropicalis* n.sp. Female. A. Capitulum and scutum, dorsum. B. Capitulum and coxae, venter. C. Hypostome. D. Spiracular plate (A = anterior; D = dorsal). E. Metatarsus and tarsus, leg I. F. Metatarsus and tarsus, leg IV.

0.63 for the paratypes. In ventral view, the basis is constricted at the middle, transverse sutural line present. Auriculae as large, flattened, posterolaterally directed protuberances. Plate on palpal segment 1 small and inconspicuous.

*Hypostome.* Shape as figured. Principal denticles 4/4 in the apical half, then 3/3 almost to the base. Lateral denticles large, medians progressively smaller to the middle. Length of toothed portion about 0.48. Missing in the paratypes.

*Scutum.* Length and width, holotype, 1.47 by 1.21; paratypes, 1.24 by 1.04 and 1.24 by 1.01, respectively. In the holotype and one of the paratypes the lateral carinae are mild elevations extending from the scapulae to about the mid-length of the scutum. In the other paratype the carinae are scarcely discernible. Cervical grooves shallow, first convergent and then divergent, not reaching the posterolateral margins. Punctations numerous and rather evenly distributed, smaller in the median field.

*Legs.* Moderate in size and length, crazed. Coxa I with a moderately long internal spur. Each coxa with a short external spur. No trochantal spurs. Tarsi abruptly narrowed subterminally. The lengths of the tarsus and metatarsus of legs I and IV of the holotype are 0.79, 0.50, 0.68, and 0.55, respectively. The corresponding leg segments of the paratypes are about 0.69, 0.41, 0.62, and 0.46.

*Spiracular plate.* Subcircular, longer axis transverse. Greatest diameter 0.35 (holotype), 0.31 (paratypes).

*Genital aperture.* Between coxae IV in the holotype; at the level of the intervals between coxae III and IV in the paratypes.

Male and immature stages unknown.

This new species resembles *I. boliviensis* Neumann (*I. bicornis* Neumann, syn.) superficially but differs in several respects. The hypostome is broader basally, more tapered, and the lateral denticles are blunter than in *I. boliviensis*. The spurs of coxa I are shorter and the internal spur is not as slender as in the latter species. The spur on coxa IV is small but definite instead of negligible or absent as in *I. boliviensis*. Furthermore, the posterior field of the scutum of the new

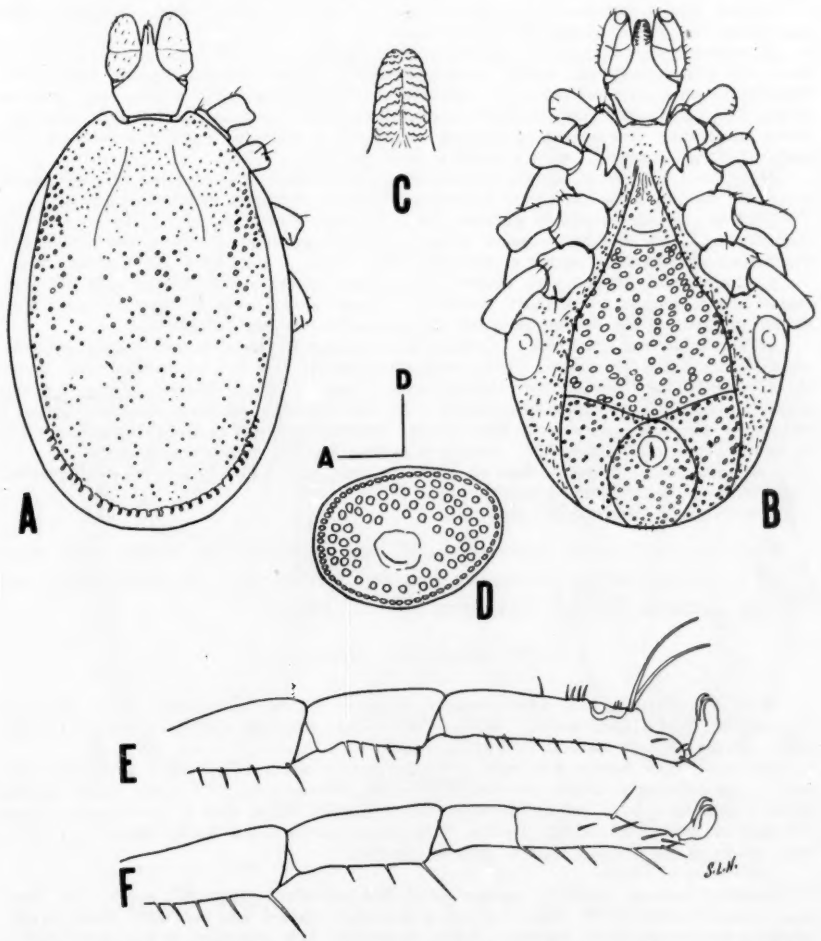


FIG. 7. *Ixodes nectomys* n.sp. Male. A. Capitulum and scutum, dorsum. B. Capitulum, coxae, and ventral plates, venter. C. Hypostome. D. Spiracular plate (A = anterior; D = dorsal). E. Metatarsus and tarsus, leg I. F. Metatarsus and tarsus, leg IV.

species lacks the conspicuous group of large punctations characteristically present in *I. boliviensis*.

That the host relationships of *I. tropicalis* and *I. boliviensis* may be different is suggested by the fact that both collections of the former are from rodents. *I. boliviensis* attacks a wide variety of hosts but has never been found on rodents so far as I am aware.

*Ixodes nectomys* n. sp.
(Fig. 7)

*Holotype.* Male, from Hacienda Cadena, Marcapata, Cusco, Peru, 1000 m. elev., November 14, 1948, C. Kalinowski. RML 31447. Host: *Nectomys squamipes garleppi*, a water rat, CNHM 65690. In the Chicago Natural History Museum.

*Description of male.*

Length, tips of scapulae to posterior margin of body, 1.77; width, 1.14. Suboval, widest just behind the middle. Color, yellow brown.

*Capitulum.* Length, tips of palpi to posterior margin of basis, 0.42; greatest width of basis, 0.27. Basis narrowed behind, posterior margin straight; cornua absent. Palpi short, broad, and with many short fine hairs; segments 2 and 3 about equal in length, their combined length, 0.25. In ventral view, the basis is short, broader in front, and with a mild ridge laterally (suggesting cornua) and continuing posteriorly to form the rounded salient posterior margin. Palpal segment 1 with a small ventral plate.

*Hypostome.* Shape as figured. Denticles as crenulations or mild teeth arranged in 10 transverse rows on each half of the hypostome. Length of toothed portion about 0.13.

*Scutum.* Surface essentially glabrous but a few very short fine hairs evident under high magnification. Lateral carinae absent, or only faintly suggested. Pseudoscutum not evident. Punctations numerous and rather evenly distributed, larger in the lateral and median areas.

*Ventral plates.* Median plate almost twice as long as the anal plate and with numerous coarse punctations and a few short, fine hairs. Adanal plates wider in front and with hairs similar to those on the median plate but the punctations smaller.

*Legs.* Rather long and slender. Coxae with a few long fine hairs; surface crazed; posterior edges of II, III, and IV salient. Coxa I with short internal spur and no external spur; coxae II, III, and IV each with a short external spur, a similar though broader internal spur on coxae II and III. Trochantal spurs absent. All tarsi abruptly narrowed distally. Tarsus I with a pair of unusually long dorsal setae situated immediately distad to Haller's organ. Length of tarsus I, 0.68; metatarsus, 0.37. Length of tarsus IV, 0.61; metatarsus, 0.43.

*Spiracular plate.* Shape as figured. Greatest dimension, 0.28. Longer axis longitudinal.

*Genital aperture.* Situated between coxae III.

Female and immature stages unknown.

The characters of the hypostome and legs, especially the notably long setae distad to Haller's organ, distinguish this new species from the other South and Central American males of this genus thus far known.

*Ixodes* (*Exopalpiger*) *andinus* n.sp.
(Fig. 8)

*Holotype.* Female, from Quilcayhuanca, Huaraz, Ancash, Peru, 4000 meters elevation, February 20, 1954, C. Kalinowski. RML 33581. Host: *Phyllotis darwini*, a rodent, CNHM 81219. In the Chicago Natural History Museum.

*Paratypes.* One female, 5 nymphs, 7 larvae, data as above; 1 nymph, 1 larva with host, locality, and collector as above, elevation 3050 meters, February 11, 1954, RML 33580, CNHM 81202; 2 nymphs, 1 larva, off *Hesperomys sorella*, CNHM 81286, data as for holotype except February 19, 1954, RML 33582. Female, 3 nymphs, 5 larvae in the Rocky Mountain Laboratory; others in the Chicago Natural History Museum.

*Description of female.*

Length of holotype (slightly engorged) including capitulum, about 3.07; width, 1.29. Body ovoid, widest in front of the middle. Color of capitulum, scutum, and legs, light yellow brown. Marginal grooves distinct. Paratype female, apparently fully engorged, is long-oval, slightly wider behind; size 7.69 by 4.80. Body dorsally and ventrally with numerous short, fine, pale hairs.

*Capitulum.* Length from tips of palpi to posterior margin of basis, 0.63. Capitulum much

widened basally by the palpigers (palpal trochanters) which are fused with the basis. Posterior margin between the palpigers convex. Porose areas large, subcircular, situated close together and close to the posterior margin. Cornua absent. Palpal segment 1 plate-like and fused with the palpiger; segments 2 and 3 fused, their combined length about 0.44. In ventral view the basis is a little constricted posterior to the level of the palpigers, posterior margin of basis broadly convex. Auriculae indicated as mild lateral extensions.

*Hypostome.* Shape as figured. Dentition 2/2 with the lateral denticles a little larger than the medians. Length of toothed portion about 0.24.

*Scutum.* Size, holotype, 1.26 by 0.98; paratype, 1.26 by 0.91. Shape as figured. No lateral carinae. Cervical grooves as a pair of elongated, divergent depressions separated from the anterior border by a distance of about half their length. Scapulae blunt. Punctations numerous, large, deep, and rather evenly distributed. Hairs few, short, and fine.

*Legs.* Long and slender and with a few short hairs. Coxae unarmed, notably large, flattened, and membraneous posteriorly (syncoxae of Schulze, 1935). The posterior membraneous portion (subcoxa of Schulze) becomes plainly evident as a whitish area when the tick is removed from alcohol and allowed to dry. Coxa I with a broad anterior process which embraces the basis capituli collar-wise and is seen from above. No trochantal spurs. Tarsi tapered gradually. Length of tarsus I, 0.57; metatarsus, 0.43. Length of tarsus IV, 0.54; metatarsus, 0.46.

*Spiracular plate.* Ovoid, longest axis transverse. Greatest length about 0.24.

*Genital aperture.* Between coxae III.

*Nymph.* With the characters of the female. Scutum proportionally a little broader, being about 0.52 long by 0.47 wide. Hypostome broken off in all specimens at hand.

*Larva.* Resembles the female and nymph. Palpigers not as well developed as in the nymph. Scutum broader than long (about 0.31 by 0.37) and without punctations. No specimens with complete hypostomes are available.

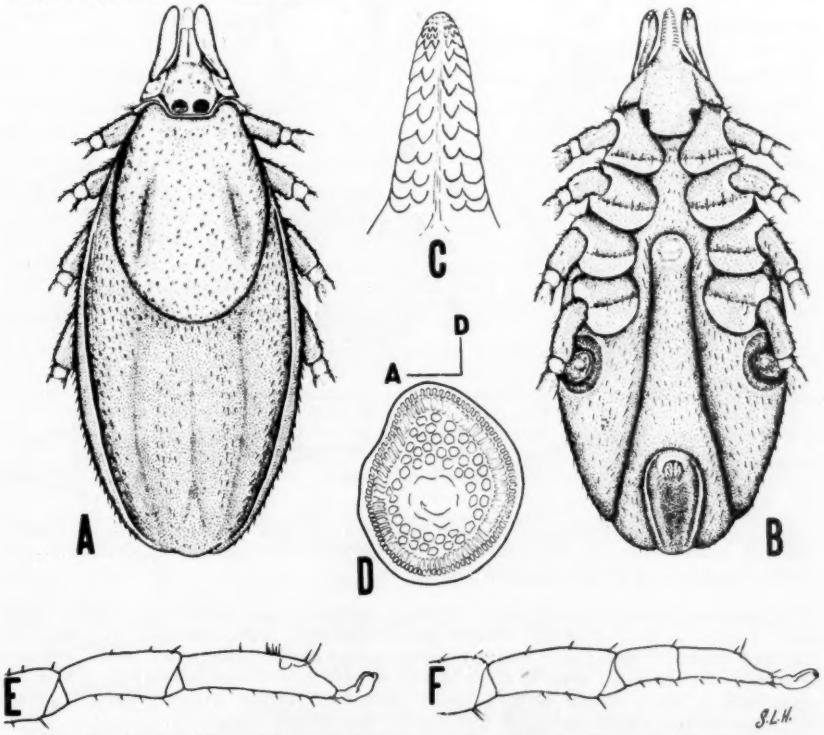


FIG. 8. *Ixodes* (*Exopalpiger*) *andinus* n.sp. Female. A. Dorsum. B. Venter. C. Hypostome. D. Spiracular plate (A = anterior; D = dorsal). E. Metatarsus and tarsus, leg. I. F. Metatarsus and tarsus, leg IV.

This, the first species of the subgenus *Exopalpiger* Schulze, 1935 to be discovered in the Western Hemisphere, is distinguished at once from all other New World species of *Ixodes* by several characters including the well developed palpigers and the unarmed, large, flattened, posteriorly membraneous coxae.

The other species that have been assigned to this subgenus are *I. priscicollaris* Schulze (type), New Guinea, *I. fecialis* Warburton and Nuttall, Australia, *I. vestitus* Neumann, Australia, *I. alluaudi* Neumann, Africa, and *I. triangulicep*s Birula, Europe.

*Ixodes chilensis* n.sp.
(Fig. 9)

*Holotype.* Female, from "Angol, Chile, 1929." RML 18706. Host: unknown. In the U. S. National Museum.

*Description of female.*

The specimen, apparently almost fully engorged, is about 7.59 long by 4.80 wide and about equally rounded at both ends; widest at the middle. Color of capitulum, scutum, and legs, yellow brown. Body dorsally and laterally with numerous fine hairs; venter with fewer, smaller hairs.

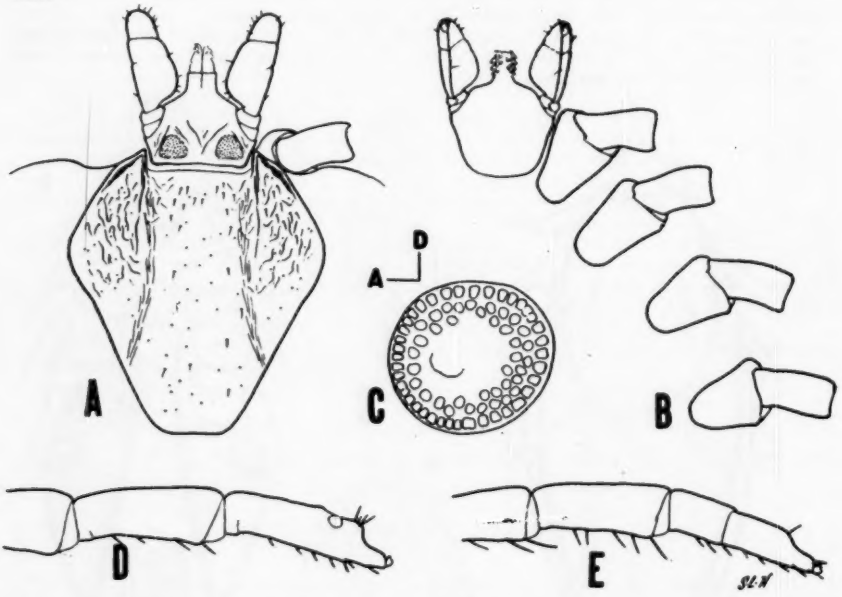


FIG. 9. *Ixodes chilensis* n.sp. Female. A. Capitulum and scutum, dorsum. B. Capitulum and coxae, venter. C. Spiracular plate (A = anterior; D = dorsal). D. Metatarsus and tarsus, leg I. E. Metatarsus and tarsus, leg IV.

*Capitulum.* Length, tips of palpi to posterior margin of basis, about 0.75; width of basis, 0.56. Surface of basis irregular. Porose areas moderately large, depressed, well separated. Posterior margin of basis nearly straight, salient; cornua absent. Palpi short, widest near base of segment 2. Segment 2 about twice as long as segment 3; combined length, 0.41. In ventral view, basis is broad, broadly rounded behind. Transverse sutural line very faint, or absent. Auriculae absent. Palpal segment 1 with a very small ventral plate.

*Hypostome.* Distal portion broken off in only specimen available. In the remaining portion the denticles are arranged 3/3, then 2/2 basally. Lateral denticles much larger than the medians.

*Scutum.* Length, 1.44; width, 1.26. Shape as figured. Surface of anterolateral areas rugose, punctate. Anterolateral margins mildly elevated. Lateral carinae absent. Cervical grooves as broad shallow curved depressions reaching to the posterolateral margins of the scutum. Punctations distinct in the median and posterior areas, indistinct or absent elsewhere. Hairs few, small, and inconspicuous.

*Legs.* Moderate in length and size. No coxal spurs. Tarsi abruptly narrowed subterminally. Length of tarsus I, 0.66; metatarsus 0.53. Length of tarsus IV, 0.59; metatarsus, 0.53.

*Spiracular plate.* Shape as figured. Greatest dimension 0.19.

*Genital aperture.* Situated at the level of the intervals between coxae II and III.

Male and immature stages unknown.

*Ixodes chilensis* resembles the North American species *I. texanus* Banks, 1909, *I. hearlei* Gregson, 1941, *I. marxi* Banks, 1908, and *I. rugosus* Bishopp, 1911, but is readily separated by its hypostome and scutum as well as by other morphological characters.

## SUMMARY

Described are *Ixodes guatemalensis,* ♀, off *Sciurus g. griseoflavus,* San Marcos, Guatemala; *I. tecpanensis,* ♀, off *Orthogeomys grandis,* Chimaltenango, Guatemala; *I. pomerantzi,* ♂, ♀, off *Sylvilagus brasiliensis,* Huancabamba, Piura, Peru, and *Sylvilagus floridanus chiapensis,* Sacatepéquez, Guatemala; *I. tapirus,* ♀, off *Tapirus pinchaque,* San Agustín, Huila, Colombia; *I. tropicalis,* ♀, off *Thomasomys aureus,* Valdivia, Antioquia, Colombia, and *Dactylomys boliviensis,* Sandia Prov., Puno, Peru; *I. nectomys,* ♂, off *Nectomys squamipes garleppi,* Marcapata, Cusco, Peru; *I.* (*Exopalpiger*) *andinus,* ♀, nymph, larva, off *Phyllotis darwini* and *Hesperomys sorella,* Huaraz, Ancash, Peru; *I. chilensis,* ♀, unknown host, Angol, Chile. The holotype of all but *I. chilensis* are in the Chicago Natural History Museum. The holotype of *I. chilensis* is in the U. S. National Museum. Paratypes of *I. tecpanensis, I. pomerantzi, I.* (*E.*) *andinus,* and *I. tropicalis* are in the Rocky Mountain Laboratory.

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# *NEOSCHÖNGASTIA MOUCHETI*, N. SP. AND *N. BRENNANI* CROSSLEY AND LOOMIS FROM AFRICA (ACARINA: TROMBICULIDAE)

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Only one bird-chigger, *Neoschöngastia mirafra* Radford, 1942, has been recorded from Africa. This species, described from material off the lark, *Mirafra africana-tropicalis* taken in Uganda, was inexcusably omitted from the key by Brennan (1951). Two other species, one of which is new and is described here, may now be added to the African fauna.

The material which serves as the basis for this report was submitted for identification by Dr. R. Taufflieb, Institut d'Études Centrafricaines, Brazzaville, French Equatorial Africa.

*Neoschöngastia brennani* Crossley and Loomis, 1955

My identification of this species was confirmed by both Mr. Crossley and Dr. Loomis. The record, based on 14 specimens off the guinea fowl, *Numida meleagris*, Tchad, North French Equatorial Africa, June 1955, J. Mouchet, collector, while not amazing, is, at least geographically, noteworthy. This is the first report of the occurrence of *N. brennani* since it was described and recorded from several species of birds (*Melanerpes erythrocephalus, Tyrannus tyrannus, Chondeste grammacus, Muscivora forficata, Calamospiza melanocorys*) from Kansas and Colorado, U.S.A.

*Neoschöngastia moucheti*, n. sp.
(Fig. 1)

*Type data:* Holotype and 21 paratypes, larvae, RML No. 33467, from the partridge, *Francolinus s. squamatus*, Cameroun, French Equatorial Africa, 7 June 1955, J. Mouchet, collector.

Holotype and some paratypes in the collection of the Rocky Mountain Laboratory. Paratypes also in the United States National Museum, the British Museum (Natural History), the South Australian Museum, the Museum of the Belgian Congo, and the University of Kansas.

*Diagnosis:* Related to *N. fullbergae* Brennan from which it is separated by 3 genualae I (2 in *fullbergae*), more and shorter dorsal setae, more and smaller setules of the sensillae, and more plumose and shorter scutal setae.

*Body:* Broad ellipsoidal. Length and width of holotype, slightly engorged, 371 by 250 microns. Eyes large, 2/2, on a plate. Anus at level of the fifth row of ventral setae.

*Gnathosoma:* Cheliceral bases, capitular sternum and palpal femoral plate with few, but large, puncta. Blade of chelicera with small tricuspid cap. All palpal setae, except the nude dorsotibial, with long branches. The femoral has many branches, the genual and ventrotibial have 5 to 7, and the laterotibial 2 or 3. Palpal claw trifurcate. Palpal tarsus with 6 or 7 branched setae and a tarsala. Galeal seta with few small branches.

*Scutum:* Roughly trapezoidal, wider than long, with rounded corners. Large puncta sparsely distributed. Striae on posterior half form a geminate pattern of concentric arches and whorls as figured. Sensillary bases widely separated, a little closer to anterior than posterior margin; a thick, cuticular fold anterior to, and sometimes concealing, them. Sensillae nearly round, the distribution of setules on anterior and posterior surfaces of head similar, *i.e.* a broad, median band of large setules along the longitudinal axis, and much smaller peripheral and basal setules which continue part way down the stem. Scutal setae plumose, the branches long. Scutal measurements of holotype: AW-52, PW-64, SB-45, ASB-21, PSB-24, AP-31, AM-34, AL-39, PL-37, S-31.

*Legs:* All segments sparsely punctate.

*Specialized setae:* Leg I—3 genualae, microgenuala; 2 nearly identical tibialae, microtibiala; short tarsala, microtarsala, subterminala, no parasubterminala, pretarsala. Leg II—genuala; 2 similar tibialae; tarsala shorter than tarsala I, microtarsala, pretarsala. Leg III—genuala; tibiala.

*Branched setae:* None of these very long, and for the most part, branches long and erect. Leg I—coxa 1; trochanter 1; basifemur 1; telofemur 5; genu 4; tibia 8; tarsus 18. Leg II—coxa 1; trochanter 1; basifemur 2; telofemur 4; genu 3; tibia 6; tarsus 15. Leg III—coxa 1; trochanter 1; basifemur 2; telofemur 3; genu 3; tibia 6; tarsus 12.

All tarsi terminated by a pair of claws and a long, slender empodium between.

*Body setae:* Dorsal setae with shorter and more appressed branches than scutal setae, 24 to 38 microns long, the length decreasing from anterior to posterior rows. Dorsal formula: 2-10-8-8-6-6-4-2. Ventral setae 2-2 (sternals) plus about 32, those posterior to the anus similar to the dorsal setae.

This species has been named for its collector, Mr. J. Mouchet, medical entomologist, Office de la Recherche Scientifique et Technique Outre Mer, Yaoundé, Cameroun, French Equatorial Africa.

SUMMARY

Two bird-chiggers are recorded from French Equatorial Africa. *Neoschöngastia moucheti* n. sp. off partridge, *Francolinus squamatus,* is described. *N. brennani* Crossley and Loomis, off guinea fowl, *Numida meleagris,* is reported for the first time since its discovery in Central United States.

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## RESEARCH NOTE

### A CASE OF DRACONTIASIS IN A NEW ENGLAND DOG

A 3-inch portion of a nematode parasite was extracted from a necrotic ulcer on the forelimb of a 10-year-old male mongrel terrier in September 1955 (by C. F. B.), and forwarded to the Angell Memorial Animal Hospital for identification. The fragment was recognized by one of us (C. W. S.) as the anterior end of a female *Dracunculus* sp. Portions of another worm had been removed from the chest wall of the same dog by another veterinarian several months before. The dog had been kenneled in Dunstable, Massachusetts, and had not been away from that vicinity during its lifetime. It had been permitted to roam freely over its owner's property, much of which is low, swampy ground.

This is apparently the seventh reported case of dracontiasis in an American dog, and the second report of this parasite in any species from New England.

Previous reports of canine infection in the United States have come from South Dakota (Benbrook, 1940, J. Am. Vet. Med. Ass. **96:** 260–263), North Carolina (Dikmans, 1948, Proc. Helm. Soc. Wash. **15:** 39–40), Texas and Iowa (Turk, 1950, J. Am. Vet. Med. Ass. **117:** 215–216), Illinois (Farmer and Witter, 1952, J. Small Anim. Med. **1:** 174), and Missouri (Elder, 1954, J. Am. Vet. Med. Ass. **124:** 390–391).

*Dracunculus* infection also has been noted in the raccoon, mink, fox, muskrat, skunk, otter and weasel in Texas, Iowa, Nebraska, Minnesota, Wisconsin, New York, New Hampshire and Maryland (Chandler, 1942, Am. J. Trop. Med. **22:** 153–157; Dikmans, 1948; Benbrook, 1932, J. Am. Vet. Med. Ass. **81:** 821–824; Cheatum and Cook, 1948, Cornell Vet. **38:** 421–423; Benbrook, 1940; Goble, 1942, J. Mammal. **23:** 221; Siegler, 1946, J. Mammal. **27:** 179).—CALVIN W. SCHWABE, *Department of Tropical Public Health, Harvard School of Public Health, and Angell Memorial Animal Hospital, Boston, Massachusetts;* HANS MEIER, *Department of Pathology, Angell Memorial Animal Hospital, Boston, Massachusetts;* AND CLARENCE F. BENT, *Nashua, New Hampshire.*

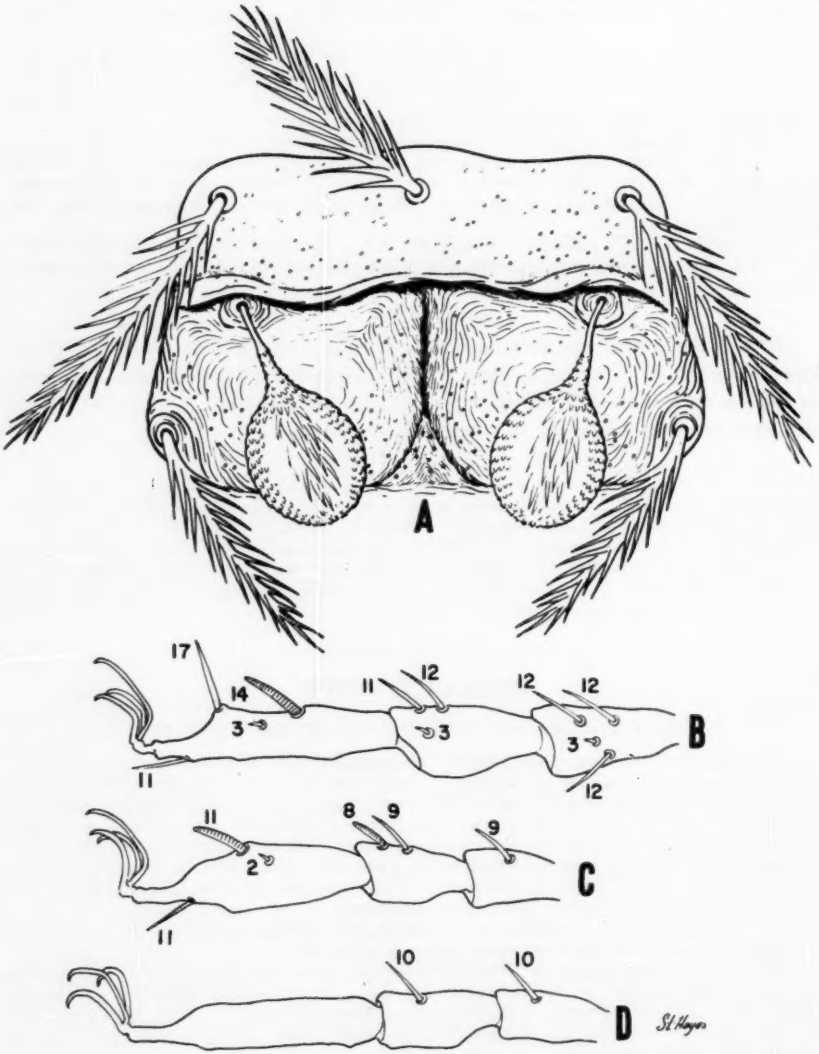


FIGURE 1. *Neoschöngastia moucheti,* new species. A. Scutum. B–D. Diagrams showing lengths in microns of specialized setae of legs I–III.

# THE NYMPHAL STAGES OF PNEUMONYSSUS SIMICOLA BANKS, 1901 (ACARINA: HALARACHNIDAE)*

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It has been pointed out (Innes *et al,* 1954) that the occurrence of mites in the lungs of experimental monkeys is probably very widespread, even though it may go unrecognized in some cases, or may be confused with other diseases. This is in spite of numerous publications on the subject by both physicians and acarologists.

Little is known about the life cycle of the lung mites. It was thought that no nymphal stages existed in the family HALARACHNIDAE (Oudemans, 1935; Newell, 1947), or in the genus *Pneumonyssus* (Vitzthum, 1931). Nymphs have been mentioned (Banks, 1901; Duncan, 1920; Dormanns, 1930a and 1930b), but Oudemans, 1935, believed that these were cases of mistaken identifications.

Nymphal stages of *Pneumonyssus simicola* have been found, however, and are described and figured here.

## MATERIAL AND METHODS

Monkey lung mites, *Pneumonyssus simicola* Banks, 1901, were encountered in rhesus monkeys, *Macaca mulatta,* at the Naval Medical Research Institute in 1953.[1] A large number of females and larvae, and a few males were recovered from the lungs of autopsied monkeys. In addition, a number of live larvae (but no adults) were recovered from living monkeys, using a tracheobronchial washing technic (Lee *et al,* 1954).

Some mites, both larvae and females, were obtained alive at autopsy from monkey lungs. Several of these were placed in slants or plates of agar and kept in a 37° C incubator. The agar had been prepared with the addition of a 0.03M concentration of ethylenediamine tetra acetic acid (EDTA) and 5% fresh monkey blood taken from living monkeys. The EDTA prevented contamination of the agar by molds or bacteria.

## OBSERVATIONS

Among the mites kept on agar, none survived for more than about 4 days. One of the larvae held on agar for 4 days before mounting developed to a protonymph within the larval skin (Fig. A). A second larva, which was obtained from a tracheobronchial washing and held in normal saline for 1 or 2 days at room temperature, also developed a protonymph within the larval skin.

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* The opinions or assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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[1] I wish to thank Drs. S. N. Stein and R. E. Lee* of the Physiology Division, and Dr. R. B. Williams, Jr., of the Pathology Division, Naval Medical Research Institute, for making this material available, and for further support from time to time. (* Present address: Mayo Clinic, Rochester, Minnesota.)

A third larva, also from a tracheobronchial washing and held in normal saline at room temperature, remained alive for 4 or 5 days. During this time, a very small quantity of detergent (Duponol ME Dry) was added to the saline for a period of about 18 hours during the second afternoon and night.[2] On the morning of the third day, the larva was returned to normal saline, slightly diluted with distilled water. On the afternoon of the third day, this larva was observed to have developed a fourth pair of legs within the opisthosoma. On the morning of the fourth day the larva had molted into an 8-legged stage, with the larval skin still adhering to the venter of the mite. On the afternoon of the fifth day, slight movement of the legs could still be seen. The adhering larval skin was carefully teased away from the mite. On the morning of the sixth day, the nymph (Figure B) and larval skin were mounted in Hoyer's solution.

*Protonymph Within Larval Skin*

Description (from 2 specimens, 1 of which is represented in Figure A). GNATHOSOMA: Palpi with 4 segments, 3 setae on distal segments and at least 1 seta on each of other 3 segments; 10 hypostomal teeth in a single longitudinal or irregular double row; 2 pairs of gnathosomal setae plus 1 pair of slightly raised knobs which appear to be setal bases. Chelicercae simple, broadly tapering to lightly sclerotized tips. LEGS: Legs I to III developed within larval legs I to III, shorter and relatively stouter than larval legs; legs IV curled within larval opisthosoma. All tarsi with a pair of short, curved, bluntly pointed claws. All setae of legs short, stout, sharply pointed. IDIOSOMA: Length 400 to 420 microns, width 164 microns. All setae short, stout, sharply pointed. VENTER: Sternal and anal plates absent. Three pairs of sternal setae, 1 pair of ventral setae on opisthosoma, 1 pair of paraanal setae and 1 postanal seta. Postanal cribrum present. DORSUM: Dorsal plate absent. 8 pairs of dorsal setae. Stigmata located laterodorsally between and above coxae III and IV.

*Free Nymph*

Description (from 1 specimen, represented in Figure B). GNATHOSOMA: Deutonymphal structures visible within protonymphal gnathosoma, both being similar to gnathosoma of protonymph within larval skin. Hypostomal teeth of both proto- and deutonymphal gnathosomas in single, longitudinal rows. Within the deutonymphal gnathosoma there appears to be the outlines of a pair of partially formed male chelicerae. These are not shown in the drawing. LEGS: Legs relatively stout. Each leg exhibits a double set of setae, those of the duetonymph within those of the protonymph. Claws of proto- and deutonymph, plus claws of adult, visible in at least one of each pair of tarsi I through IV. The adult claws are internally located in each tarsus, and cannot be confused with tarsal setae. IDIOSOMA: Length 384 microns, width 240 microns. All setae short, stout, sharply pointed. VENTER: Sternal and anal plates absent. The sternal setae are in 2 sets, one set (outer) for the protonymph and one (under protonymphal skin) for the deutonymph. It is difficult to place the ventral setae on the opisthosoma because the protonymphal skin has been partly cast off dorsally, and is gathered in folds on the ventral side behind legs IV. This accounts for the comparatively numerous setae on the ventral opisthosoma of Figure B. It seems evident, however, from examining the slide-mounted specimen under the phase contrast microscope, that only 1 pair of ventral setae normally appear on the opisthosoma, both for the proto- and deutonymph. One pair of para-anal setae and 1 postanal seta, and a postanal cribrum are present for both proto- and deutomymph. DORSUM: (Deutonymph) Dorsal plate absent. Eight pairs of dorsal setae. Two stigmata on each side, one pair (deutonymphal?) located laterodorsally between and above coxae III and IV, and the second pair (protonymphal?) located just posterior and slightly ventral to the first pair. The outline of a male genital opening may be seen ventrally (and subcutaneously) between the first set of sternal setae. A narrow tube leads posteriorly for a short distance from this opening, as shown in the drawing.

[2] The amount of detergent added was not determined, but it was sufficient to wet the larva so it would not adhere to the surface film of the saline. The integument of both larvae and adults is normally hydrophobic.

## DISCUSSION

The nymphal mites considered here were obtained under unnatural conditions. It is possible that under natural conditions in the host, the nymphal stages have been either eliminated entirely, or occur so rapidly that they have been overlooked. The fact that the specimen from which Figure B was taken seems to show protonymphal, deutonymphal, and adult characters may lend support to the latter possibility.

There is an item of interest in this connection in Oudemans, 1935. He includes a description of the larva of *Pneumonyssus duttoni* Newstead and Todd, a species found in the lungs of a Guenon monkey (*Cercopithecus schmidti*) from the Upper Congo. A drawing of the larval venter is included (p. 501) which shows an extra leg, curled within one side of the opisthosoma posterior to the third pair of legs. This extra leg is in a position similar to the left leg IV of Figure A of this paper. There is no explanation of this extra leg in Oudemans' description of the larva or elsewhere in the text, however.

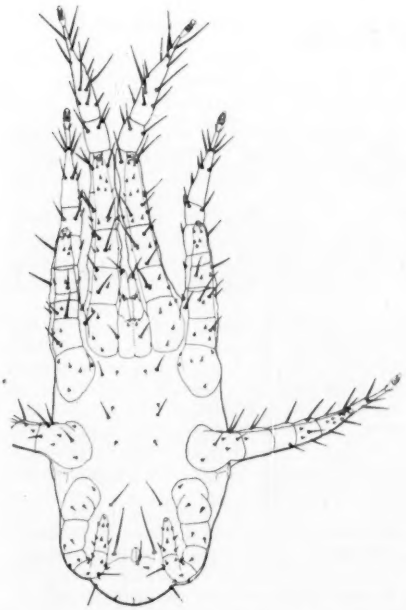

FIGURE A. Protonymph within larval skin.

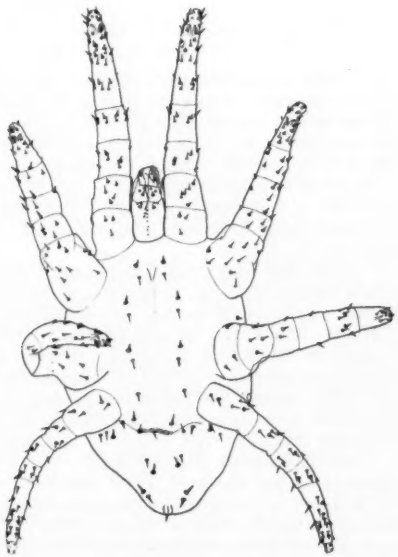

FIGURE B. Free nymph.

## SUMMARY

The proto- and deutonymphal stages of the monkey lung mite, *Pneumonyssus simicola* Banks, 1901 (host: *Macaca mulatta*) are described and figured. It is believed that these are the first descriptions of nymphal stages for any of the mite family HALARACHNIDAE to appear in the literature.

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## RESEARCH NOTES

### OBSERVATIONS ON THE POSSIBILITY OF DEVELOPING A STRAIN OF *TRICHINELLA SPIRALIS* RESISTANT TO RADIATION

In 1949, Alicata and Burr (Science **109:** 595–596) pointed out for the first time the possible use of radioactive cobalt in irradiating trichinous pork so as to break the life cycle of the parasite and in that way to assist in the control of human infections. Subsequent studies were conducted by the writer (1951, J. Parasitol. **37:** 491–501) to determine the radiation dosages necessary to induce certain destructive or lethal action on the parasite. This report is based on observations that were made during 1951. It is presented at this time because of the increased interest in the use of radiation in pork for the control of human trichinosis. In recent years, several additional papers have been published on this subject by Gould and co-workers (1955, Am. J. Path. **31:** 933–963).

In the 1951 report, the writer pointed out that the degree of injury produced on the egg cells of individual female worms at a given dosage of radiation was not uniform; that whereas in some cases most of the egg cells within the uterus were destroyed, a few underwent normal cleavage. It was then thought that such conditions might have been brought about by (1) a variation in the amount of radiation received by the individual egg cells, or (2) the fact that some cells were more resistant to radiation than others. If the latter assumption were correct, one would suppose that following radiation only the more resistant cells would develop. In such a case it would be conceivable that by irradiating the parasites through several generations, one might gradually build up a strain highly resistant to radiation.

The experiments reported below were designed to explore the above possibility. The following method was used: Fresh trichinous rodent muscle, not exceeding 5 mm in thickness, was wrapped in cellophane and exposed to a dose of 5,000 r. This dosage was chosen because from previous observations of the writer (1951; *loc. cit.*), it produced complete sterility in about one-half of the female parasites. In this way it was possible to insure adequate propagation of the parasites and sufficient number of larvae in the musculature of the host animal for subsequent infections of other animals. The X-ray unit used in these studies was operated at 100 KV (7 ma.; FSD, 15.5 cm.; HVL, 1.35 mm. al.; inherent filter, 0.5 mm. al.; 320 r/min.). Following radiation, the muscle was fed to about 10 young rats. Six days following infection, 4 of the rats were killed to determine the percentage of sterile adult female trichinae in the intestinal tract. The other rats were killed about 5 weeks after infection, and the muscle tissue containing the encysted larvae was irradiated as before and fed to other rats. The above procedure was followed from one generation to another. The criterion used for determining resistance to radiation was the sterility noted among the irradiated adult female worms. It was believed that if resistance did develop, the females in subsequent generations would show increasingly less sterility than those of the parent generation irradiated for the first time.

TABLE I. Percentage of sterility observed among adult female trichinae following irradiation (5,000 r) of the larval stage in each successive generation

| Rat Number | Parent Generation | First Generation | Second Generation | Third Generation | Fourth Generation | Fifth Generation | Sixth Generation | Stock[1] Strain |
|---|---|---|---|---|---|---|---|---|
| 1 | 18 | 9 | 65 | 0 | 84 | 70 | 69 | 62 |
| 2 | 83 | 10 | 70 | 1 | 82 | 66 | 75 | 72 |
| 3 | 48 | 15 | 76 | 1 | 86 | 59 | 59 | 60 |
| 4 | 38 | 13 | 53 | 0 | 84 | 61 | 60 | 50 |
| Average | 46.7 | 11.7 | 64.0 | 0.5 | 84.0 | 64.0 | 65.7 | 61.0 |

[1] The stock strain constituted normal non-previously irradiated parasites maintained in laboratory animals and was of the same origin as that used in the parent generation. The muscle containing larvae of the stock strain was irradiated together with the muscle containing the larvae of the sixth generation.

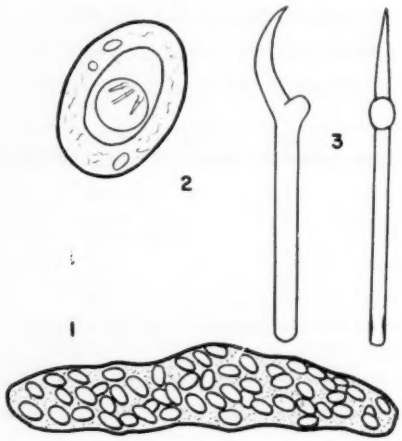
The results of the above study, which was carried out through 6 parasite generations, are presented in Table I. The table shows the percentage of sterility noted among the female trichinae irradiated for the first time (parent generation) and that among the subsequent generations. It is noted that, although the average percentage of sterility varied considerably in the different generations, there was no clear evidence that resistance to radiation had progressively increased. It is not clearly understood why the percentage was very low in the first and third generations. The percentage of sterility noted in the sixth generation (65.7 per cent) was approximately similar to that obtained from parasites of the stock strain (61.0 per cent) which were irradiated for the first time.

The above data seem to indicate that the difference in the failure or ability of individual egg cells in trichinae to develop following irradiation is more likely due to the amount of radiation received rather than to the difference in resistance to radiation. This failure to develop a strain of trichinae resistant to radiation is similar to the recent findings of Gould and co-workers (1955; *loc. cit.*) in which they report that irradiation of larvae with cobalt-60 through several generations did not induce in them the property of radioresistance. (This investigation was supported in part by Grant E-104 from the U. S. Public Health Service. The irradiation of trichinous meat reported in this paper was carried out with X-ray facilities available at the Tripler General Hospital, Honolulu, under the direction and supervision of Col. A. O. Haff and the assistance of Chief C. K. Lare).—JOSEPH E. ALICATA, *University of Hawaii, Honolulu, Hawaii.*

## *HYMENOLEPIS SPHENOMORPHUS* FROM *SOREX CINEREUS* IN ALASKA

Following the description of *Hymenolepis sphenomorphus* from *Sorex v. vagrans* (Locker and Rausch 1952, J. Washington Acad. Sci. **42**: 26–31) Voge (1955, J. Parasit. **41**: 74–76) recorded the same species from *Sorex vagrans* in California. Voge and Rausch (1955, J. Parasit. **41**: 566–574) have reported this tape from *S. obscurus* and *S. cinereus* near Anchorage, Alaska. However, in studying specimens of this cestode from *Sorex cinereus* collected from the Tanana River drainage near Fairbanks and from the North fork of the Huslia River, 66° North, 156°50′ West, the writer observed a sizeable variation in the reported mature gravid proglottids and eggs.

Unlike the material observed by Voge, the gravid proglottids of *H. sphenomorphus* are devoid of any reproductive organs, normally unattached, and much larger in size. These "egg cases" (Fig. 1) are bluntly rounded on both ends measuring 370–780 microns (average 644) by 110–190 microns (average 144). They contain from 30 to 90 eggs each and have been observed intact in the rectal portion of the digestive tract. In 92% of the specimens examined




the cirrus had disappeared by the time the sac was detached from the strobila. It is interesting to note that in only 5 proglottids of 73 worms examined was a spined terminal knob observed, whereas both Locker and Rausch and Voge indicate that the evaginated cirrus with terminal knob is the rule rather than the exception.

Because the dehydration process usually distorts the eggs beyond recognition, the following observations were made from fresh and formalin-fixed material. The eggs (Fig. 2) are typically ovoid averaging 55 microns long by 41 microns wide and containing 3 cell membranes. The first 2 membranes are difficult to separate; however, if the egg is somewhat dehydrated the thin membrane immediately below the shell pulls away clearly revealing three separate membranes surrounding the oncosphere (the first two membranes are indicated as a single line in Fig. 2). The substance separating the second membrane from the third membrane is hyaline material with highly refractive "fat globules" interspersed throughout. Nothing corresponding to the protoplasmic strands of the dibothriocephalid embryophore was observed. However, "protoplasmic" strands and comma-shaped cellular inclusions are located in the cytoplasmic material separating these two membranes. Within the inner membrane and surrounding the oncosphere is a very granular yolk-like material much less dense than the oncosphere. The three pairs of hooklets (Fig. 3) show no measurable variation in size or shape; each measures from 9 microns to 10.5 microns in length. There is a prominent guard separating the blade from the shaft, but no terminal knob was observed in a lateral view; neither was there observed a measurable difference in the thickness of any one pair of hooks.—T. T. DUNAGAN, *Arctic Aeromedical Laboratory, APO 731, Seattle, Washington.*

## CORRECTION FACTORS FOR FECAL CONSISTENCY IN MAKING NEMATODE EGG COUNTS OF SHEEP FECES

In interpreting fecal egg counts, the consistency of the feces, which is an index of its water content, should be considered. According to T. K. Ewer and D. P. Sinclair (1951. New Zeal. J. Sci. and Techn., Sec. A **32:** 35–48), workers in Australia and New Zealand multiply sheep fecal egg counts by the following figures, depending on fecal consistency: normal pellets, 1; soft formed (pellet formation beginning), 4/3; soft (no formation, sticky), 2; very soft (mushy to thick liquid), 3; diarrheic (liquid), 4. According to W. H. Southcott (1955. Austral. J. Agric. Res. **6:** 456–465), the following correction factors were developed by Gordon for sheep feces: normal feces (pellets), 1.0; soft pellets and soft formed, 1.5; unformed, 2.0; very soft and diarrheic, 3.0–4.0. However, the water content data on which these correction factors were based were not given.

In connection with a study of the gastrointestinal nematodes of sheep, it was felt desirable to determine independently the water content of feces of different consistencies. Fecal samples were taken directly from the rectum of 69 sheep grazing on an alfalfa-rye pasture, their consistency was recorded, and 3-gram portions were weighed out. These were then dried for two hours in a hot air oven at 160° C, weighed again, and their dry weights were calculated as percentages of their wet weights.

The dry weights of 26 normal, pelleted fecal samples varied from 19 to 40%, with a mean of 29.3% and a standard deviation of 6.02%. The dry weights of 17 soft-pelleted fecal samples ranged from 14 to 27%, with a mean of 21.0% and a standard deviation of 3.73%. The dry weights of 26 soft-formed fecal samples ranged from 12 to 25%, with a mean of 18.6% and a standard deviation of 3.16%. No very soft or diarrheic stools were studied.

Using Student's $t$ test, $t$ was found to be 2.21 in comparing the dry weights of the soft-formed and soft pelleted samples, 4.97 in comparing the dry weights of the soft-pelleted and normal-pelleted samples, and 7.87 in comparing the dry weights of the soft-formed and normal-pelleted samples. The first figure indicates significance beyond the 5% level, the second beyond the 0.1% level, and the third far beyond the 0.1% level.

On the basis of the above figures, and using a mean dry weight of 29.3% for normal, pelleted feces, the correction factors for the soft-formed samples were calculated to range from 1.2 (for the sample with 25% dry matter) to 2.4 (for the sample with 12% dry matter), with a mean of 1.6. The correction factors for the soft-pelleted fecal samples ranged from 1.1 (for the sample with 27% dry matter) to 2.1 (for the sample with 14% dry matter), with a mean of 1.4. The correction factors for the normal-pelleted samples ranged from 0.7 (for the sample with 40% dry matter) to 1.5 (for the sample with 19% dry matter); the mean, of course, was 1.0.

Perhaps the most interesting finding was the wide variation in dry matter of fecal samples which appeared grossly to have about the same consistency. This variation was sufficient to make a two-fold difference in egg counts possible, even if correction factors were applied. This is therefore another source of variability in fecal egg counts.

Despite the wide variation encountered in each group, the foregoing statistical analysis indicates that it is worthwhile to apply correction factors to fecal egg counts. However, the use of different factors for soft-formed and soft-pelleted feces is not justified. The best correction factor for both these types of feces is 1.5. This agrees with Gordon's figure, cited above. Without this factor, the range of variation in the present study would be 3.4-fold instead of two-fold.—NORMAN D. LEVINE AND DAVID T. CLARK,* *College of Veterinary Medicine and Agricultural Experiment Station, Univ. of Illinois, Urbana, Illinois.* (* Present address, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan.)

## OBSERVATIONS ON THE INFECTIVITY OF *ECHINOCOCCUS* EGGS OBTAINED FROM FOXES (*ALOPEX LAGOPUS* LINN.) ON ST. LAWRENCE ISLAND, ALASKA

Eggs of the alveolar form of *Echinococcus* are quite resistant to arctic temperatures and remain viable for prolonged periods of time, even under the most inclement conditions of cold. Eggs, in tap-water, stored for 2½ years at 2° C were infective to ground and tree squirrels (*Citellus tridecemlineatus* Mitchill; *C. franklini* Sabine; *Sciurus carolinensis* Gemlin), white and field mice (*Microtus* spp.). Very old dehydrated fox scats, abundantly dispersed over the tundra, were randomly collected, stored at room temperature for over 2 years and were found to contain viable eggs infective to voles. Under favorable natural conditions, *Echinococcus* eggs probably remain infective for several years. Johnson (1951, Science in Alaska; 125–131) observed the microclimate beneath the snow for red back mice, red squirrels and weasels to range from 15–25° F, even when the air temperature was – 40°, – 50° F or below. The high percentage of infected St. Lawrence Island field mice (Thomas *et al*, 1955, Science **120:** 1102–1103) distant from the vicinities of fox dens leads one to infer that such microclimates might also exist for *Echinococcus* eggs. Schiller (1955; J. Parasitol. **41:** 578–582) reports a vole infected with eggs subjected to – 51° C. He further points out that the climate of St. Lawrence Island "rarely goes as low as – 37° C." It is conceivable that such eggs shed prior to the onset of winter seldom are exposed to the direct adversities of this season and consequently do not have to be protected from severe cold. The epidemiological nature of alveolar echinococcosis, however, is undoubtedly enhanced by the resistance of the eggs to sub-freezing temperatures which has contributed to its spread across the arctic—LYELL J. THOMAS AND BERT B. BABERO, *Zoology Laboratory of the University of Illinois and the Arctic Aeromedical Laboratory.*

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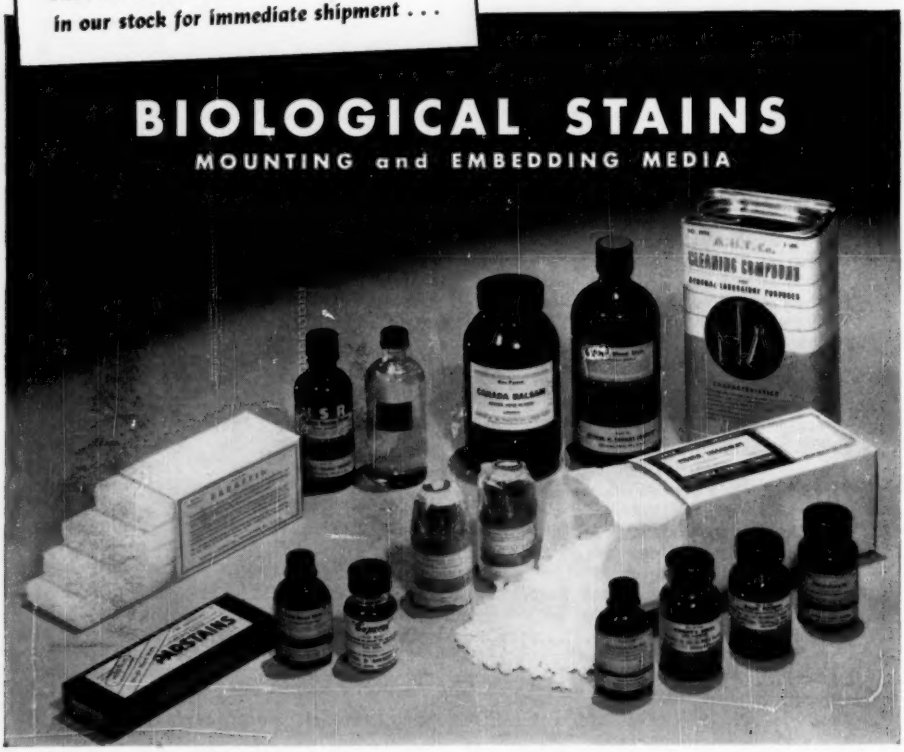


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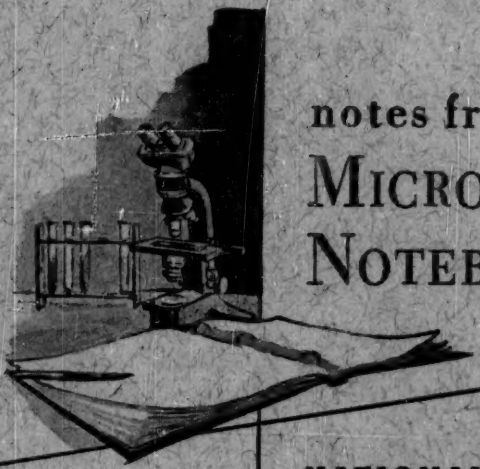

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